PATENT COOPERATION TREAT

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
Sim & McBurney
Attn. RAE, Patricia
330 University Avenue
6th floor
Toronto, Ontario M5G 1R7
CANADA

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

Date of mailing *(day/month/year)*	06.02.98
Applicant's or agent's file reference: 8322-3 PAR	**PAYMENT DUE** within 45 ~~months~~/days from the above date of mailing
International application No.: PCT/CA 97/00568	International filing date *(day/month/year)*: 13/08/1997

Applicant

UNIVERSITY TECHNOLOGIES INTERNATIONAL INC. et al.

1. This International Searching Authority

(i) considers that there are 3 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

2-6,9,10; 1,8,12-20 in part

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited**, within the time limit indicated above, to pay the amount indicated below:

DEM 2200,- (Fee per additional invention) x 2 (number of additional inventions) = DEM 4400,- (total amount of additional fees)

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest**, i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [X] Claim(s) Nos. See attached sheet (third page) have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Véronique Baillou

Form PCT/ISA/206 (July 1992)

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/CA 97/00568

1. Claims: 2-6, 9, 10; 1,8,12-20 in part

Peptides of claims 2-6, and subject-matter relating to said peptides.

The present application does not comply with the requirements of unity of invention [Rule 13(1) PCT]. At least 3 separate inventions have been identified. Each of them is characterised by an individual "special technical feature"; there is no technical interrelation between these inventions (see below). The applicants are therefore asked to pay additional search fees. Otherwise the International Search Report will be limited to the first invention specified below [Rule 40 PCT; Art. 17(3)(a) PCT].

Searched:
1st: Claims 2-6, 9,10; 1, 8, 12-20 in part: peptides of claims 2-6, and subject-matter relating to said peptides.

Not yet searched:
2nd: Claims 11; 12-20 in part: the peptide of claim 11, and subject-matter relating to it.

3rd-nth: Claims 1, 7, 8, 12-20 in part: Other peptides of claim 1, and subject-matter relating to said peptides.
NOTE: Formula 1 covers a vast range of structurally almost unrelated peptides; it can be assumed that thousands of peptides correspond to the vague criteria of claim 1. Therefore, applicants are asked to indicate themselves substructures (= putative invention(s)) which are linked by a unifying non-trivial structural feature. One search fee will be due for each invention. Simple payment for this item, without indication of the structure(s) to be searched will result in further non-unity objections, and/or an incomplete search.

The following arguments reflect the preliminary opinion of the ISA concerning unity of invention:

1). Rule 13(2) PCT demands that "Rule 13.1 PCT shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression 'special technical features' shall mean those technical features which de fine a contribution which each of the claimed invention considered as a whole makes over the prior art."
The Administrative Instructions under the PCT, Annex B(f)(v) state that "if it can be shown that at least one Markush alternative is not novel, unity of invention should be re considered",
and the PCT Preliminary Examination Guidelines C-III 7.6 say that "if the common matter of the independent claim is well known, and the remaining subject-matter ... differs without there being any unifying novel concept common to all of them, then clearly there is lack of unity".

1.1. As outlined in the description, the problem underlying the present application is to provide peptide with anti-anaphylactic (-endotoxic, -inflammatory) activity.

1.2. The solution to this problem in its broadest form is represented by the general formula of claim 1.

1.3. A vague general formula as such is not regarded as the unifying concept. A novel and non-obvious "special technical feature" must be common to all embodiments falling under that formula.
NOTE that the compound of claim 11 does not even correspond to the vague structural criteria of claim1.

1.4. Even for the restricted scope of the first invention, several compounds have been retrieved that destroy the novelty of a preferred range of compounds, see all "X" citations of the preliminary international search report.
It follows that the vague structural features of claim 1 cannot be used to motivate unity of invention.

1.5. Short anti-anaphylactic peptides with structural features that overlap with the scope of claim 1 (especially if seen together with the compound of claim 11!) are known, see for example the general claim of WO-A-92/11858. The concept "short anti-anaphylactic peptides" can thus not be used to motivate unity of invention.
[Moreover, the structural formula of claims 1 overlaps with classes of peptides which are distinguished by completely different biological activities, see for example Enkephalin derivatives (cf. as an example the compound H-2890 , and page 203 of the BACHEM citation). It is therefore not credible that all compounds covered by claim 1 can have the same biological activity.]

1.6. The submandibular glands have been discussed for years as the origin of factors that downregulate neutrophil function. The origin of the peptides can thus not be used to motivate unity of invention, see Comp. Biochem. Physiol. vol 106C, page 44.

2). Please note also that Rule 13 PCT has a regulatory function (to prevent unjustified saving of fees and to ensure ready comprehensibility). Also from this more pragmatic approach the present application lacks unity of invention:
First, due to the lack of constant characteristic structural elements, competitors cannot inform themselves readily on the existing situation regarding protective rights.
Second, the equitable levying of fees has to be respected. Because of its heterogeneous content, the present application entails a far greater than average expense in the procedure up to grant (keep in mind that there is an ample background concerning compounds of related structure and function, thus necessitating several independent searches of restricted scope).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 13-18

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claims 13-18 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

An[illegible]o Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

[illegible]ternational Application No
PCT/CA 97/00568

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
1-6,8-10,12-20
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.
3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.
4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 11858 A (ABBOTT LABORATORIES) 23 July 1992 * claim 1, claim 11; pages 1-4 *	1-6, 8-10, 12-20
Y	MATHISON, R. ET AL.: "Neural regulation of neutrophil involvement in pulmonary inflammation" COMP. BIOCHEM. PHYSIOL., vol. 106c, no. 1, 1993, page 39-48 XP002051005 * page 44-45, chapter "Submandibular gland ..." *	1-6, 8-10, 12-20
X	"Bachem Feinchemikalien AG, Hauptstrasse 144, CH-4416 Bubendorf/Switzerland:" BACHEM CATALOG S13, 1993, page 203 and 533 XP002051006	1,3,7-9
A	* compounds H-2585, H-2590; H-5125 and page 533 in general *	1-6, 8-10, 12-20
X	ABDERHALDEN, E. ET AL.: "Über das Verhalten von Tetrapeptiden ..." FERMENTFORSCHUNG, vol. 16, 1942, pages 98-114, XP002051007 * p. 99; p. 109, p. 11 *	1,3,7,8

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Form PCT/ISA/206 (Annex, first sheet) (July 1992)

International Application No
PCT/CA 97/00568

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SLOOTSTRA, J.W.: "Structural aspects of antibody-antigen interaction ..." MOLECULAR DIVERSITY, vol. 1, 1996, pages 87-96, XP002051008 * figs 2-4 * ---	1-4,6-8
P,X	MATHISON, R.D. ET AL.: "Submandibular glands: novel structures ..." CAN. J. PHYSIOL. PHARMACOL., vol. 75, 1997, pages 407-413, XP002051009 * whole disclosure * -----	1-5,7,8, 12-20

Patent Family Annex

Information on patent family members

International Application No
PCT/CA 97/00568

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9211858 A	23-07-92	US 5386011 A	31-01-95
		AT 148891 T	15-02-97
		CA 2095359 A	28-06-92
		DE 69124705 D	27-03-97
		EP 0564588 A	13-10-93
		JP 6504055 T	12-05-94

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 8322-3 PAR

Box No. I TITLE OF INVENTION

PEPTIDES FOR TREATMENT OF INFLAMMATION AND SHOCK

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

UNIVERSITY TECHNOLOGIES INTERNATIONAL INC.
Suite 204, 609 - 14 Street NW
Calgary, Alberta, Canada
T2N 2A1

☐ This person is also inventor.

Telephone No.
(403) 270-7027

Facsimile No.
(403) 270-2384

Teleprinter No.

State *(i.e. country)* of nationality:
CA

State *(i.e. country)* of residence:
CA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

MATHISON, Ronald
208 Silverhill Crescent NW
Calgary, Alberta, Canada
T3B 3Y3

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:
CA

State *(i.e. country)* of residence:
CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

RAE, Patricia
Sim & McBurney
330 University Avenue, 6th Floor
Toronto, Ontario, Canada
M5G 1R7

Telephone No.
(416) 595-1155

Facsimile No.
(416) 595-1163

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

DAVISON, Joseph S.
600 Dalmeny Hill NW
Calgary, Alberta, Canada
T3A 1R3

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: CA

State *(i.e. country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

BEFUS, Dean
14015 Valleyview Drive
Edmonton, Alberta, Canada
T5R 5T9

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: CA

State *(i.e. country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

MOORE, Graham
1762 1A Avenue NW
Calgary, Alberta, Canada
T2N 0B2

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: CA

State *(i.e. country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked):*

Regional Patent

☒ **AP ARIPO Patent:** **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line):*

☒ **AL** Albania .....
☒ **AM** Armenia .....
☒ **AT** Austria .....
☒ **AU** Australia .....
☒ **AZ** Azerbaijan
☒ **BA** Bosnia and Herzegovina
☒ **BB** Barbados
☒ **BG** Bulgaria .....
☒ **BR** Brazil .....
☒ **BY** Belarus
☒ **CA** Canada
☒ **CH and LI** Switzerland and Liechtenstein
☒ **CN** China .....
☒ **CU** Cuba .....
☒ **CZ** Czech Republic .....
☒ **DE** Germany .....
☒ **DK** Denmark .....
☒ **EE** Estonia .....
☒ **ES** Spain .....
☒ **FI** Finland .....
☒ **GB** United Kingdom
☒ **GE** Georgia .....
☒ **HU** Hungary .....
☒ **IL** Israel .....
☒ **IS** Iceland
☒ **JP** Japan .....
☒ **KE** Kenya .....
☒ **KG** Kyrgyzstan .....
☒ **KP** Democratic People's Republic of Korea .....
☒ **KR** Republic of Korea .....
☒ **KZ** Kazakstan .....
☒ **LC** Saint Lucia
☒ **LK** Sri Lanka
☒ **LR** Liberia
☒ **LS** Lesotho .....
☒ **LT** Lithuania
☒ **LU** Luxembourg .....
☒ **LV** Latvia
☒ **MD** Republic of Moldova .....
☒ **MG** Madagascar .....
☒ **MK** The former Yugoslav Republic of Macedonia .....
☒ **MN** Mongolia
☒ **MW** Malawi .....
☒ **MX** Mexico .....
☒ **NO** Norway
☒ **NZ** New Zealand .....
☒ **PL** Poland .....
☒ **PT** Portugal .....
☒ **RO** Romania
☒ **RU** Russian Federation .....
☒ **SD** Sudan
☒ **SE** Sweden
☒ **SG** Singapore
☒ **SI** Slovenia .....
☒ **SK** Slovakia .....
☒ **TJ** Tajikistan .....
☒ **TM** Turkmenistan .....
☒ **TR** Turkey .....
☒ **TT** Trinidad and Tobago .....
☒ **UA** Ukraine .....
☒ **UG** Uganda .....
☒ **US** United States of America .....
☒ **UZ** Uzbekistan .....
☒ **VN** Viet Nam .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

☒ ZW. Zimbabwe.....
☒ SL. Sierra Leone.....
☒ GH. Ghana.....
☒ YU. Yugoslavia.....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) GB	13 August 1996 (13-08-96)	9617021.2	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA/_____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year):* Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	5 sheets
2. description	:	30 sheets
3. claims	:	3 sheets
4. abstract	:	1 sheets
5. drawings	:	15 sheets
Total	:	54 sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify):*

Figure No. 11 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Patricia A. Rae
Sim & McBurney

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

09/051395
-SOCO

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
8322-3 PAR		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 97/00568	13/08/1997	13/08/1996
Applicant		
UNIVERSITY TECHNOLOGIES INTERNATIONAL INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [X] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00568

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/08 C07K5/10 C07K7/06 A61K38/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 11858 A (ABBOTT LABORATORIES) 23 July 1992 * claim 1, claim 11; pages 1-4 * ---	1-6, 8-10, 12-20
Y	MATHISON, R. ET AL.: "Neural regulation of neutrophil involvement in pulmonary inflammation" COMP. BIOCHEM. PHYSIOL., vol. 106c, no. 1, 1993, page 39-48 XP002051005 * page 44-45, chapter "Submandibular gland ..." * --- -/--	1-6, 8-10, 12-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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Date of the actual completion of the international search	Date of mailing of the international search report
22 December 1997	30.04.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	HERMANN R.

Form PCT/ISA/210 (second sheet) (July 1992)

Inte ional Application No
PCT/CA 97/00568

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Bachem Feinchemikalien AG, Hauptstrasse 144, CH-4416 Bubendorf/Switzerland:" BACHEM CATALOG S13, 1993, page 203 and 533 XP002051006	1,3,7-9
A	* compounds H-2585, H-2590; H-5125 and page 533 in general *	1-6, 8-10, 12-20
X	ABDERHALDEN, E. ET AL.: "Über das Verhalten von Tetrapeptiden ..." FERMENTFORSCHUNG, vol. 16, 1942, pages 98-114, XP002051007 * p. 99; p. 109, p. 11 *	1,3,7,8
X	SLOOTSTRA, J.W.: "Structural aspects of antibody-antigen interaction ..." MOLECULAR DIVERSITY, vol. 1, 1996, pages 87-96, XP002051008 * figs 2-4 *	1-4,6-8
P,X	MATHISON, R.D. ET AL.: "Submandibular glands: novel structures ..." CAN. J. PHYSIOL. PHARMACOL., vol. 75, 1997, pages 407-413, XP002051009 * whole disclosure *	1-5,7,8, 12-20

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/CA 97/00568

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13-18
because they relate to subject matter not required to be searched by this Authority, namely:
see separate sheet

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see separate sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

claims : 2-6, 9, 10; 1,8,12-20 in part

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

1. Claims: 2-6, 9, 10; 1,8,12-20 in part

Peptides of claims 2-6, and subject-matter relating to said peptides.

Searched:
1st: Claims 2-6, 9,10; 1, 8, 12-20 in part: peptides of claims 2-6, and subject-matter relating to said peptides.

Not yet searched:
2nd: Claims 11; 12-20 in part: the peptide of claim 11, and subject-matter relating to it.

3rd-nth: Claims 1, 7, 8, 12-20 in part: Other peptides of claim 1, and subject-matter relating to said peptides.
NOTE: Formula 1 covers a vast range of structurally almost unrelated peptides; it can be assumed that thousands of peptides correspond to the vague criteria of claim 1. Therefore, applicants are asked to indicate themselves substructures (= putative invention(s)) which are linked by a unifying non-trivial structural feature. One search fee will be due for each invention. Simple payment for this item, without indication of the structure(s) to be searched will result in further non-unity objections, and/or an incomplete search.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

Claims Nos.: 13-18

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claims 13-18 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ... Application No
PCT/CA 97/00568

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9211858 A	23-07-92	US 5386011 A	31-01-95
		AT 148891 T	15-02-97
		CA 2095359 A	28-06-92
		DE 69124705 D	27-03-97
		EP 0564588 A	13-10-93
		JP 6504055 T	12-05-94
		PT 99940 A	29-01-93

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(54) Title: PEPTIDES FOR TREATMENT OF INFLAMMATION AND SHOCK

(57) Abstract

Submandibular peptides have been isolated and characterised and provide pharmaceutical compositions for the treatment or prevention of anaphylactic reactions, endotoxic reactions and SIRS-induced shock.

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PEPTIDES FOR TREATMENT OF INFLAMMATION AND SHOCK

Field of the Invention

This invention relates to peptides which modulate anaphylactic, endotoxic and inflammatory reactions in mammals.

Background of the Invention

In the description which follows, references are made to certain literature citations which are listed at the end of the specification.

Infections, trauma (e.g. falls, automobile accidents, gun and knife wounds), cardiovascular accidents (e.g. aneurysms and ischemic events often associated with surgery) and endogenous inflammatory reactions (e.g. pancreatitis and nephritis) often lead to profound dysfunction of the homeostatic mechanisms involved in regulating cardiovascular and immune system function.

Several conditions such as ischemia and infections, through inappropriate or excessive activation of the immune system, may result in cardiovascular dysfunction that develops over a period of hours to days. Compromised cardiovascular function increases morbidity and is life threatening.

Systemic inflammatory response syndrome (SIRS) is diagnosed largely on observed physiological changes such as increase (fever) or decrease in body temperature (hypothermia), increased heart rate (tachycardia), increased respiration rate (tachypnea), elevated or diminished white blood cell counts and inadequate perfusion of tissues and organs. Changes in blood pressure are not included in the definition because they occur late in the course of the syndrome. Decreases in blood pressure reflect the development of shock, and contribute to multiple organ failure, a leading cause of death in these patients. This definition of sepsis

syndrome includes a large number of patients who exhibit similar physiological signals but have no evidence of any type of infection; other insults which induce a sepsis-like condition include pancreatitis, burns, ischemia, multiple trauma and tissue injury (often due to surgeries and transplants), haemorrhagic shock and immune-mediated organ dysfunction.

SIRS is the 13th leading cause of death in the United States of America. On average, 40% of sepsis syndrome patients are dead within 28 days of admission to intensive care.

The standard therapies for SIRS and septic shock involve administration of antibiotics to bring the infection under control and fluid/colloid therapy to maintain circulating blood volume. Frequently, drugs which help maintain blood pressure, such as dopamine and vasopressin, are also administered.

Recent strategies for developing more targeted therapies for the treatment of sepsis have been disappointing. In addition, many molecules in the new generation of anti-septic agents are very expensive and most possibly produce untoward immunological and cardiovascular reactions which make them contra-indicated in some cases of non-bacteremic shock.

Hypersensitivity to environmental antigens can, in severe cases, result in anaphylactic shock, which is treated by the administration of fluids and vasoactive agents to restore blood pressure.

There remains a need for inexpensive and effective agents for treatment of cardiovascular shock, sepsis, systemic inflammatory response syndrome and anaphylaxis.

The salivary glands are classically viewed as accessory digestive glands which mediate their actions through exocrine secretion, although appreciation has grown recently for the importance of their endocrine secretions (1-3). The exocrine secretion of biologically active peptides from the salivary glands is essential for the health of the mouth (4), whereas the endocrine

secretions of these glands help maintain the structure and function of a large variety of internal tissues and organs such as the digestive tract (5-7), the mammary glands (8), the liver (9,10), and the reproductive tract (11,12). Rosinski-Chupin et al. (13,14) have described a protein from the male rat submandibular gland which is androgen-regulated and which they believed to have a male-specific function in the rate.

Another important action of salivary endocrine secretions is the modulation of the immune system, with effects being observed on lymphocyte (15,16), mast cell (17) and neutrophil (18,19) functions. The submandibular glands also regulate inflammatory reactions associated with the late-phase pulmonary inflammation induced by allergen in sensitized rats (20-22), and their removal exacerbates the severity of the hypotensive responses induced by intravenously administered lipopolysaccharide (LPS) (23). Removal of the submandibular glands, however, does not affect arterial blood pressure in the normal state (23).

In accordance with one embodiment, the invention provides a peptide of the formula: $R^1 - X^1 - X^2 - R^2$

wherein X^1 is an aromatic amino acid residue;

X^2 is any amino acid residue;

R^1 is NH_2 - or an amino acid sequence $X^3 - X^4 - X^5$

wherein X^3 is an aliphatic amino acid residue having a side chain hydroxyl group and

X^4 and X^5 are the same or different and are any amino acid residue and

wherein R^2 is a sequence of 1 to 3 amino acid residues which are the same or different and are aliphatic amino acid residues.

In accordance with a further embodiment, the invention provides a peptide having the amino acid sequence

Ser-Gly-Glu-Gly-Val-Arg (Sequence ID NO:1).

In accordance with a further embodiment, the

invention provides a method for treating or preventing SIRS-induced hypotension in a mammal comprising administering to the mammal an effective amount of a described peptide or of an effective fragment or derivative of the peptide.

In accordance with a further embodiment, the invention provides a method for treating or preventing anaphylactic hypotension in a mammal comprising administering to the mammal an effective amount of a described peptide or of an effective fragment or derivative of said peptide.

In accordance with a further embodiment, the invention provides a method of reducing or preventing an anaphylactic reaction in a mammal comprising administering an effective amount of a described peptide or of an effective fragment or derivative of said peptide to the mammal.

In accordance with a further embodiment, the invention provides a method of reducing or preventing an endotoxic reaction in a mammal comprising administering an effective amount of a described peptide or 11 or an effective fragment or derivative of said peptide to the mammal.

In accordance with a further embodiment, the invention provides a method for treating an inflammatory disorder in a mammal comprising administering to the mammal an effective amount of a described peptide or of an effective fragment or derivative of the peptide to the mammal.

In accordance with a further embodiment, the invention provides an antibody which specifically recognises an epitope of a peptide of the invention.

In accordance with a further embodiment, the invention provides a method of determining the peptide SGP-T or the peptide SGPS in a biological fluid comprising obtaining a sample of the biological fluid and determining the peptide in the fluid by immunoassay employing an antibody which specifically epitope of a

peptide of the invention.

Certain embodiments of the invention are described, reference being made to the accompanying drawings, wherein:

Figure 1 shows the effect of SGP-T concentration on the average decrease over a 1 hour period in mean arterial blood pressure (MABP) induced by intravenous LPS (*Salmonella typhosa*, 3.5 mg/kg), evaluated in pentobarbital anesthetized unoperated (■) and sialadenectomized (▲) rats. * $p < 0.05$

Figure 2 shows the average decrease in MABP in rats (sensitised 1 month previously with 3000 larvae of the nematode *Nippostrongylus brasiliensis*) challenged by injection of worm antigen (100 worm equivalents), after the following treatments:

A: no drug

B: 10 µg SGP-T

C: 35 µg SGP-T

D: 100 µg SGP-T. * $p < 0.05$

Figure 3 shows % treated rats with disrupted MMC's (solid bars) or diarrhoea (hatched bars) after the indicated pretreatments, followed by challenge with egg albumin (EA) or bovine serum albumin (BSA).

Figure 4 shows number of neutrophils migrating into carrageenin-soaked sponges implanted in rats treated with various indicated concentrations of SGP-T.

Figure 5 shows superoxide anion production, expressed as n moles O_2^-/min/10^7 cells, in neutrophils from carrageenin-soaked sponges implanted in rats treated with various indicated concentrations of SGP-Tcarr = carrageenin-soaked sponge from rat with no SGP-T treatment; sal = saline soaked sponge (control) from rat with no SGP-T treatment.

Figure 6 shows superoxide anion production by human (■) or rat (▲) neutrophils treated <u>in vitro</u> with SGP-T.

Figure 7 shows the effect of SGP-T and various analogues and fragments on antigen-induced jejunal segment contraction (from ovalbumin (OA) - sensitized rats). Con : saline control; T : SGP-T; A1 : ADIFEGG (Sequence ID NO:2); A2 (Sequence ID NO:3) : TAIFEGG (Sequence ID NO:3); A3: TDAFEGG (Sequence ID NO:4); A4: TDIAEGG (Sequence ID NO:5); A5: TDIFAGG (Sequence ID NO:6); X6: TDIFE (Sequence ID NO:7); X7: TDIFEGG-NH2 (Sequence ID NO:8); X8: FEGGG (Sequence ID NO:9). Ure/OA Ratio (X axis) is the ratio of the contractile response to the cholinergic agonist, urecholine (URE), divided by the contractile response to OA. * $P < 0.05$. The dotted line indicates the control (Con) response across the figure.

Figure 8 shows the effect of SGP-T and various analogues on antigen - induced jejunal segment contraction (from OA - sensitized rats). Cont : control; T : SGP-T; FEG : FEG; Sar : FE-Sarcosine; CIT : FE - Citrulline; Pro : FE - proline. Ure/OA Ratio (X axis) is the ratio of the contractile response to the cholinergic agonist, urecholine (URE), divided by the contractile response to OA. * $P < 0.05$. The dotted line indicates the control (Con) response across the figure.

Figure 9 shows the effect of SGP-T and various analogues on antigen-induced anaphylactic hypotension. X axis : ventricular peak systolic pressure (VPSP). Con : saline control; feG : D-phenylalanine, D-glutamate, glycine. * $P < 0.05$

Figure 10 shows the effect of feG on antigen-induced anaphylactic hypotension. X axis : VPSP

Figure 11 shows the incidence of disrupted intestinal myoelectric activity (disrupted MMC's) and diarrhoea in rats treated with egg albumin alone (EA), bovine serum albumin alone (BSA), SGP-T prior to OA (SGP-T), FEG prior to OA (FEG), feG prior to OA (feG) and feG orally prior to OA (feG oral).

Figure 12 shows changes in ventricular peak systolic pressure (VPSP) over time in rats treated with saline

(■), 10 µg/kg SGP-T (▲) or 100 µg/kg SGP-T (◆) prior to challenge with antigen. * $P < 0.05$, $n \geq 4$

Figure 13 shows the antigen-induced decrease in VPSP in rats after pre-treatment with various doses of SGP-T. * $P < 0.05$.

Figure 14 shows MABP 10 min before (Before) or 60 minutes after (After) LPS injection in rats treated 30 minutes before LPS with FEG (hatched bars) or vehicle (open bars).

Figure 15 shows the effect of SGP-T at various concentrations (solid bars) on endotoxin-induced fever (X axis: ΔTb = change in body temperature (°c)) relative to pre-endotoxin body temperaturs. Open bars: control * $P < 0.05$ $n = 4$.

Detailed Description of the Invention

The present invention relates to peptides obtained from mammalian submandibular glands which modulate anaphylactic reactions and endotoxic and inflammatory reactions in mammals.

Anaphylactic reactions in mammals are severe and harmful reactions to an allergen to which a mammal has become sensitized or hypersensitized. These reactions are severe or excessive manifestations of an immunological protective mechanism designed to protect the mammal against foreign antigens. Anaphylactic reactions can occur in response to such allergens as insect bites and stings, plant pollens, plants such as poison ivy, food allergens, such as nuts and seafood, animal dander and house dust and have a role in the etiology of asthma, rhinitis, urticaria, eczema and certain gastrointestinal disorders.

An anaphylactic reaction to an antigen can be associated with hypotension, increased intestinal motility, diarrhea , bronchial constriction and edematous swelling. Anaphylactic hypotension may be severe enough to cause anaphylactic shock, which may be life-threatening.

Endotoxic reactions in mammals develop consequent to the activation of the immune system in an attempt to rid the body of a bacterial infection. Unlike anaphylactic reactions, which develop rapidly in a matter of minutes, endotoxic reactions increase in severity over a period of days. Pro-inflammatory mediators such as cytokines, which are released as part of the body's response to an infection, stimulate the respiratory and cardiovascular systems. In some patients, the fight against the infection fails and the pro-inflammatory mediators begin to have deleterious effects on the patient, promoting the progressive collapse of the cardiovascular system, poor organ perfusion and further tissue damage. If these severe inflammatory reactions are not arrested, endotoxic shock can ensue.

A clinically useful paradigm has been developed that helps define patients that could possibly progress into sepsis or sepsis-like conditions, and eventually shock. This paradigm is called the systemic inflammatory response syndrome (SIRS).

SIRS is defined clinically as the presence of <u>two or more of the following criteria:</u>

1) a body temperature greater than 38°C or less than 36°C;

2) a heart rate greater than 90 beats/minute;

3) a respiratory rate greater than 20 breaths/minute;

4) a white blood cell count greater than 12 million/ml or less than 4 million/ml.

Some 68% of all patients entering the hospital possess SIRS, and thus are at risk for the development of sepsis or a sepsis-like condition and shock. A confirmed infectious process (i.e. positive blood cultures) are required for the rigorous diagnosis of sepsis. Nonetheless, some infection-negative patients progress to a stage of severe sepsis, which is defined by the presence by one of the following conditions:

1) a reduction of systolic blood pressure to less

than 90 mm Hg;

2) a systemic manifestation of poor tissue perfusion as reflected by lactic acidosis, low urine output or acute alteration of mental state.

An SIRS patient can progress directly to severe sepsis, in the absence of a definable infectious agent. Some of the conditions that favour progression to the severe sepsis stage include: pancreatitis, burns, and cerebral or spinal injuries. Patients with SIRS can proceed to SIRS-induced hypotension and SIRS-induced shock. A patient is considered to be in shock if he or she remains hypotensive (i.e. systolic blood pressure below 90 mm Hg) following the administration of 500 ml of fluid.

The cervical sympathetic trunk-submandibular gland (CST-SMG) axis has recently been identified as a neuroendocrine axis that modulates pulmonary inflammatory and cardiovascular responses provoked by sensitizing antigen (20,21) and endotoxins (23), respectively. The cervical sympathetic nerves modulate such inflammatory reactions, for example that induced by intravenous administration of gram negative bacterial endotoxin, by regulating the release, from the submandibular glands (3), of factors which reduce the severity of the reaction (23).

The present inventors have obtained from rat submandibular glands, and characterized, two novel peptides which have profound effects on a variety of cardiovascular and immunological perturbations and are likely candidates for the agents by which the submandibular gland exerts its influence on anaphylactic or endotoxic reactions.

In accordance with one embodiment, the present invention provides submandibular gland peptides S and T (SGP-S and SGP-T), which have the following amino acid sequences:

SGP-S: NH_2-Ser-Gly-Glu-Gly-Val-Arg-COOH (SGEGVR: Sequence ID NO:1);

SGP-T: NH_2-Thr-Asp-Ile-Phe-Glu-Gly-Gly-COOH (TDIFEGG Sequence ID NO:8).

The invention also includes effective fragments and derivatives of these peptides which retain at least one biological activity of the peptide from which they are derived. The terms "derivative" extends to any functional and/or chemical equivalent of the peptides of the invention and includes peptides having one or more amino acid substitutions; peptides incorporating unnatural amino acids and peptides having modified side chains. The invention also includes homologues of these peptides in other species, including human. Such homologues may be identified using antibodies to the peptides disclosed herein, as will be understood by those of ordinary skill in the art.

The biological effects of SGP-T include reduction or prevention of endotoxic hypotension, at doses as low as 1 μg peptide/kg body weight; reduction or prevention of anaphylactic hypotension in foreign protein-sensitised mammals; a protective effect against ventricular dysfunction during systemic anaphylaxis; attentuation of antigen-induced perturbations of gastrointestinal motility in ovalbumin-sensitised animals; a significant reduction of *in vitro* antigen-induced smooth muscle contraction, in muscle from ovalbumin-sensitized animals; a significant reduction of the fever provoked by bacterial endotoxin; an approximately 50% inhibition of neutrophil migration; a prevention of carrageenin-induced inhibition of superoxide production by phorbal myristate (PMA) and formyl-Met-Leu-Phe. (fMLP);

The invention enables pharmaceutical compositions comprising the peptide SGP-T or an effective fragment or derivative thereof, for the treatment or prevention of anaphylactic reactions, including anaphylactic shock, and endotoxic reactions, including endotoxic shock.

These compositions may also be used for the treatment or prevention of systemic inflammatory response syndrome (SIRS) and the treatment of inflammation or any

disorder ameliorated by down regulation of neutrophil function. Such disorders include rheumatic diseases, inflammatory bowel disease and post-ischemic lesions subsequent to stroke or cardiac infarct.

The peptides of the invention modulate and reduce the severity of anaphylactic reactions, including cardiovascular and intestinal anaphylactic reactions, anaphylactic shock and SIRS-induced reactions, including endotoxic shock and SIRS-induced shock.

The invention also enables methods for preventing or treating anaphylactic reactions, including anaphylactic shock, endotoxic reactions, including endotoxic shock, SIRS, inflammation and disorders ameliorated by down regulation of neutrophil function.

Examination of fragments and derivatives of SGP-T indicates that various amino acid substitutions may be effected without loss of biological activity, as shown in Tables 2, 3 and 4.

SGP-T and various full length derivatives thereof are active in inhibiting anaphylactic and endotoxic hypotension.

The fragment FEG shows the same activity as SGP-T in inhibiting anaphylactic hypotension but does not inhibit endotoxic hypotension. The anti-anaphylactic hypotension activity of FEG is maintained if D amino acids are substituted for F and G.

Although the mechanism of action of the peptides of the invention is still unknown, these results suggest that the anti-endotoxic activity and the anti-anaphylactic activity of SGP-T may be mediated by different cell receptors.

SGP-T does not prevent an initial anaphylactic reaction (see Figure 12 which shows the same initial blood pressure drop in SGP-T-treated animals and controls) but it does prevent a sustained anaphylactic reaction. This later-phase inhibitory effect could occur by one or both of two mechanisms: 1) prevention of the release of mediators whose synthesis is stimulated by the

binding of the allergen to the immunoglobulin E receptors on mast cells. Such mediators include leukotrienes, platelet activating factor and prostaglandins; 2) prevention of the reaction of either the preformed or the newly formed mediators with effector cells contributing to the hypotension or disturbances in intestinal motility. Practice of the invention is not, however, dependent on the mechanism by which these peptides act.

The invention also enables methods for preventing and/or treating inflammation, anaphylactic reactions, including anaphylactic shock, endotoxic reactions including endotoxic shock, and SIRS, by administration of an effective amount of SGP-T or an effective fragment or derivative thereof.

The invention further enables pharmaceutical compositions comprising the peptide SGP-S or an effective fragment or derivative thereof, for the treatment or prevention of endotoxic reactions, including endotoxic shock.

The invention also enables methods for preventing or treating endotoxic reactions, including endotoxic shock, by administration of an effective amount of the peptide SGP-S or of an effective fragment or derivative thereof.

Peptides SGP-T and SGP-S and effective fragments or derivatives thereof may be administered prophylactically to patients at risk of developing shock before they progress into shock, or may be administered after shock has developed, to combat hypotension.

Peptide SGP-T and effective fragments or derivatives thereof may be administered prophylactically to prevent anaphylactic reactions in susceptible subjects, for example individuals who experience anaphylactic reactions to insect bites or stings, to pollens, to particular foods or to occupational allergens such as grain dusts or latex, or used to arrest the progression of an already initiated anaphylactic reaction to a more severe or more systemic reaction.

Preparation of Peptides

Peptides in accordance with the invention or fragments or derivatives thereof may be prepared by any suitable peptide synthetic method.

Chemical synthesis may be employed, for example standard solid phase peptide synthetic techniques may be used. In standard solid phase peptide synthesis, peptides of varying length can be prepared using commercially available equipment. This equipment can be obtained, for example, from Applied Biosystems (Foster City, CA.). The reaction conditions in peptide synthesis are optimized to prevent isomerization of stereochemical centres, to prevent side reactions and to obtain high yields. The peptides are synthesized using standard automated protocols, using t-butoxycarbonyl-alpha-amino acids, and following the manufacturer's instructions for blocking interfering groups, protecting the amino acid to be reacted, coupling, deprotecting and capping of unreacted residues. The solid support is generally based on a polystyrene resin, the resin acting both as a support for the growing peptide chain, and as a protective group for the carboxy terminus. Cleavage from the resin yields the free carboxylic acid. Peptides are purified by HPLC techniques, for example on a preparative C18 reverse phase column, using acetonitrile gradients in 0.1% trifluoroacetic acid, followed by vacuum drying.

Peptides may also be produced by recombinant synthesis. A DNA sequence encoding the desired peptide is prepared and subcloned into an expression plasmid DNA. Suitable mammalian expression plasmids include pRC/CMV from Invitrogen Inc. The gene construct is expressed in a suitable cell line, such as a Cos or CHO cell line and the expressed peptide is extracted and purified by conventional methods. Suitable methods for recombinant synthesis of peptides are described in "Molecular Cloning" (25). Derivatives of a peptide may be prepared by similar synthetic methods. Examples of side chain modifications contemplated by the present invention include modification of amino groups such as by reductive

alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidation with methylacetimidate; acetylation with acetic anhydride; carbamylation of amino groups with 2, 4, 6, trinitrobenzene sulfonic acid (TNBS); alkylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2, 3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via acylisourea formation followed by subsequent derivatization, for example, to a corresponding amide.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid-, t-butyglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers or amino acids.

Examples of conservative amino acid substitutions are substitutions within the following five groups of amino acids:

Group 1: F Y W
Group 2: V L I
Group 3: HKR
Group 4: M S T P A G
Group 5: D E

Fragments or derivatives of the peptides of the invention may be screened for their effectiveness by one of the assay systems described herein. The assay selected will depend on the biological activity of interest in each case. For example, peptide fragments or derivatives may be screened for their effectiveness in inhibiting intestinal anaphylaxis by the method described

in Example 7 or for their effectiveness in inhibiting endotoxic hypotension by the method described in Example 2.

The peptides of the invention may be administered therapeutically by injection or by oral, nasal, buccal, sub-lingual, rectal, vaginal, transdermal or ocular routes in a variety of formations, as is known to those in the art.

For oral administration, various techniques can be used to improve stability, based for example on chemical modification, formulation and use of protease inhibitors. Stability can be improved if synthetic amino acids are used, such as peptides or betidamino acids, or if metabolically stable analogues are prepared.

Formulation may be, for example, in water/oil emulsion or in liposomes for improved stability. Oral administration of peptides may be accompanied by protease inhibitors such as aprotinin, soybean trypsin inhibitor or FK-448, to provide protection for the peptide. Suitable methods for preparation of oral formulations of peptide drugs have been described, for example, by Saffran et al., (26), (use of trasylol protease inhibitor); Lundin et al. (27) and Vilhardt et al., (28).

Due to the high surface area and extensive vascular network, the nasal cavity provides a good site for absorption of both lipophilic and hydrophilic drugs, especially when coadministered with absorption enhancers. The nasal absorption of peptide-based drugs can be improved by using aminoboronic acid derivatives, amastatin, and other enzyme inhibitors as absorption enhancers and by using surfactants such as sodium glycolate, as described in Amidon et al., (29).

The transdermal route provides good control of delivery and maintenance of the therapeutic level of drug over a prolonged period of time. A means of increasing skin permeability is desirable, to provide for systemic access of peptides. For example, iontophoresis can be

used as an active driving force for charged peptides or chemical enhancers such as the nonionic surfactant n-decylmethyl sulfoxide (NDMS) can be used.

Transdermal delivery of peptides is described in Amidon et al. (29) and Choi et al. (30).

Peptides may also be conjugated with water soluble polymers such as polyethlene glycol, dextran or albumin or incorporated into drug delivery systems such as polymeric matrices to increase plasma half-life.

More generally, formulations suitable for particular modes of administration of peptides are described, for example, in Remington's Pharmaceutical Sciences (31).

The particular dosage required in a given subject can be determined by the attending physician. A starting dosage in the range of 1 μg peptide / kg body weight to 100 μg / kg can be employed, with adjustment of the dosage based on the response of a particular subject, as understood by those of ordinary skill in the art.

Antibodies

The peptides of the invention may be coupled to a carrier protein to increase immunogenicity for antibody production. For example, the peptides of the invention may be coupled to bovine serum albumin or keyhole limpet haemocyanin.

In order to prepare peptides for production of polyclonal antibodies, fusion proteins containing a selected peptide, such as peptide 15 or peptide 42, can be synthesized in bacteria by expression of corresponding DNA sequences in a suitable cloning vehicle. Fusion proteins are commonly used as a source of antigen for producing antibodies. Two widely used expression systems for *E. coli* are lacZ fusions using the pUR series of vectors and trpE fusions using the pATH vectors. The peptides can then be purified, coupled to a carrier protein if desired, and mixed with Freund's adjuvant (to help stimulate the antigenic response of the animal) and injected into rabbits or other appropriate laboratory animals.

Following booster injections at weekly intervals, the rabbits or other laboratory animals are bled and their serum isolated. The serum can be used directly or the polyclonal antibodies purified prior to use by various methods including affinity chromatography.

As will be understood by those skilled in the art, monoclonal antibodies may also be produced using the peptides of the invention. A selected peptide, coupled to a carrier protein if desired, is injected in Freund's adjuvant into mice. After being injected three times over a three week period, the mice spleens are removed and resuspended in phosphate buffered saline (PBS). The spleen cells serve as a source of lymphocytes, some of which are producing antibody of the appropriate specificity. These are then fused with a permanently growing myeloma partner cell, and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as HAT. The wells are then screened by ELISA to identify those containing cells making binding antibody. These are then plated and after a period of growth, these wells are again screened to identify antibody-producing cells. Several cloning procedures are carried out until over 90% of the wells contain single clones which are positive for antibody production. From this procedure a stable line of clones which produce the antibody is established. The monoclonal antibody can then be purified by affinity chromatography using Protein A Sepharose, ion-exchange chromatography, as well as variations and combinations of these techniques. Truncated versions of monoclonal antibodies may also be produced by recombinant techniques in which plasmids are generated which express the desired monoclonal antibody fragment in a suitable host.

Human neutrophils appear to possess receptors for the peptides of the invention (see, for example, Figure 6) and it is likely that these peptides, or homologues thereof, occur in humans.

Antibodies to SGP-T and SGP-S, or their human

homologues, may be used to determine the circulating concentration of these peptides. By knowing the circulating levels of the SGP-T and SGP-S peptides, a diagnostic tool becomes available to predict the susceptibility of the patient to progress from systemic inflammatory response syndrome SIRS) to the development of septic or non-septic shock. Low circulating levels of these peptides would indicate increased susceptibility to progression from SIRS to shock, whereas high circulating levels would be indicative of reduced susceptibility to the development of shock.

In addition, knowledge of the circulating levels of SGP-T and/or SGP-S in humans may be used to monitor and adjust dosage levels of the peptides in treatment. Furthermore, this information could be used to predict alternative therapeutic approaches in the management of patients, for example treatment with antibiotics, aggressive volume replacement therapy or the administration of vasoactive agents such as dopamine or vasopressin.

In addition, determination of circulating levels of SGP-T may be useful in determining the reactivity state of neutrophils and monocytes in inflammatory conditions such as arthritis or inflammatory bowel disease. Low circulating levels may indicate the need for neutrophil suppression therapy using the peptides of the invention.

EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Example 1

Isolation, Purification and Sequencing of SGP-T and SGP-S

The purification procedures involved homogenization of rat submandibular glands in 0.1N HCL, centrifugation of the extract at 15,000g for 1h, sequential molecular weight cut-off filtration of the supernatant with Amicon 30,000, Centricon 10,000 and 3,000 filters followed by 5 steps of reverse phase high performance liquid chromatography (RP-HPLC) using 20 to 50% acetonitrile. At each step of the purification, biologically active fractions were identified by monitoring their ability to reduce LPS-induced hypotension in sialadenectomized rats. The fractions of submandibular gland extracts generated during purification were assayed by measuring endotoxin induced hypotension in sialadenectomized rates. The extracted glands were lyophilized at each step of the purification to remove acid and/or organic solvents. All fractions were dissolved in 0.9% saline (pH 7.0) at a concentration equivalent of 0.2 μg of original submandibular gland/μl, with each rat receiving 1μl/kg body weight of the extracts.

Two procedures were used to extract anti-shock activities from submandibular glands. Initially, the glands were homogenized in distilled water containing 50,000 units/ml of Aprotinin (Sigma) and 5 mM benzamidine (Sigma) to inhibit serine proteases (protease inhibitor extraction). One g of frozen submandibular gland in 5 ml of extraction medium was blended for 2 min at high speed in a Waring blender, and the mixture was disrupted further with a ground glass homogenizer. The homogenate was centrifuged at 15,000 g for 1 h with a sorval RC5C maintained at 4° C, and the supernatant, designated the crude homogenate was frozen at -70°C for subsequent purification. This crude homogenate was then fractionated using several molecular weight exclusion filtrations, which consisted of Diaflow Ultrafilters 30,000 and 10,000 (Amicon Div., W.R. Grace & Co., Danvers, MA), performed under 70 psi of nitrogen at 20°C, and Centricon 3000 filters (Amicon Div., Beverly, MA) which were centrifuged with a Sorval RC5C centrifuge at

4°C for 4 h at 7,500 g. The supernatant was then eluted through Sep-Pak columns (Waters Chromatographic Div., Millipore Corp., Milford Corp., Milford, MA) with 50% acetonitrile, and after drying the eluant was run on preparative RP-HPLC (20% to 50% acetonitrile). Further HPLC purification was performed using analytical RP-HPLC columns was performed using analytical RP-HPLC (Vydac C18, 5μm, 4.6 mm x 25 cm). The protein eluting from the HPLC columns was detected monitoring absorbance at 214nM, a wavelength which detects peptide bonds.

With the protease inhibitor extraction procedures the biological activity was not conserved consistently through to the analytical HPLC stage. This instability was probably due to the limited half-life of the kallikrein inhibitors and the rich array of proteases found in salivary glands. Thus, an acid extraction procedure was applied. The submandibular glands were homogenized in 0.1N HCl to destroy all enzymatic activity, and the extract was purified using the procedures described for the protease inhibition extraction. Using this acid extraction procedure biological activity was conserved through all steps of the purification.

The two peptides were sequenced at the Protein/Peptide Synthesis Unit of The University of Calgary and the Alberta Peptide Institute at The University of Alberta, Edmonton, Alberta.

The peptides were then synthesized, using standard solid phase synthesis Sephadex G-10 and HPLC purified and their amino acid compositions confirmed.

Example 2: Effects of SGP-T and SGP-S on Experimental Endotoxemia

The animal model of endotoxic shock used involves intravenous injection of endotoxin (3.5 mg/kg of lipopolysaccharide (LPS) from *Salmonella typhosa*) into pentobarbital - anaesthetized Sprague-Dawley rats, to produce a fall in mean arterial blood pressure within 3 to 5 minutes. The endotoxin was injected slowly over 1

min. via the jugular vein, and mean arterial blood pressure in mm mercury (MABP) was monitored continuously for 60 min, with the average blood pressure being calculated at 15, 30, 45 and 60 min. Studies were performed on unoperated rats and rats with their submandibular glands removed (sialadenectomized).

The results shown in Table 1 are the averages of three different experiments using the protocol defined above. The data shown represent the average decrease in MABP (dec MABP) and the % Decrease in MABP over the 60 minutes following LPS administration. SGP-S (100µg/kg) and SGP-T (100µg/kg) were administered 90 min. before LPS. The sialadenectomized rats exhibited a more severe hypotensive response to LPS than unoperated rats. The average MABP for the 60 minutes following LPS injection (MABPaft) was significantly less for the sialadenectomized rats (68.03±3.4 mm Hg) than for the unoperated rats (88.03±3.6 mm Hg). Neither SGP-T nor SGP-S, when given prior to the LPS challenge, had appreciable effects on MABP.

In unoperated rats, SGP-T reduced the drop in MABP elicited by LPS, and this effect was independent of time of administration of the peptide. Overall, SGP-T reduced by 60% the decrease and the percent decrease in MABP induced by endotoxin, relative to pre-LPS values. SGP-S, on the other hand, had no effect on the shock induced by endotoxin in unoperated rats.

In sialadenectomized rats, SGP-S (but not SGP-T) significantly reduced the drop in MABP after LPS, the decrease in MABP after LPS relative to MABP bef, and the percent decrease in MABP relative to MABP bef.

The dose-response relationship of the inhibitory effect of SGP-T and SGP-S on endotoxin-induced hypotension was also examined and the results are shown in Figure 1. It can be seen that SGP-T, given intravenously 1.5 hours before LPS, dose-dependently inhibited the decrease in blood pressure induced by the

LPS in sialadenectomized rats. SGP-T at doses of 1, 3.5 and 10 µg/kg significantly prevented the LPS-provoked drop in blood pressure. In contrast, saline controls (SGP-T zero) exhibited an average MABP drop of 55 mm Hg. Doses of SGP-T higher than 10 µg/kg were less effective in preventing the shock. The optimal dose of SGP-T for reducing LPS-induced hypotension in unoperated rats was higher than in operated animals.

Example 3: Effect of SGP-T and SGP-S on Anaphylaxis

Cardiovascular Anaphylaxis

The effect of SGP-T on antigen-induced anaphylactic hypotension was examined in Sprague-Dawley rats sensitized 5 weeks previously with the nematode parasite *Nippostrongylys brasiliensis*. Under pentobarbital anesthesia, worm antigen (100 worm equivalents) was injected and arterial blood pressure was followed for 1 hour. The results are shown in Figure 2. Whereas rats treated with saline vehicle (SGP-T zero) experienced a drop in blood pressure after antigen challenge of approximately 30 mm Hg, SGP-T given 10 minutes prior to induction of anaphylaxis dose-dependently protected against the anaphylactic hypotension.

Intestinal Anaphylaxis

SGP-T was also effective in preventing intestinal anaphylaxis. Figure 3 shows that in rats sensitized to the antigen hen egg albumin, instillation of the antigen (EA) into the jejunum after saline pretreatment promoted diarrhoea and disruption of normal fasting gut motility (migrating myoelectric complexes; MMCs). SGP-T, given intravenously at doses as low as 10 µg/kg significantly attenuated these anaphylactic reactions. A dose of 100 µg/kg totally prevented the manifestation of anaphylaxis.

A similar protection against intestinal anaphylaxis was observed *in vitro* using isolated intestinal (jejunal) segments obtained from egg albumin sensitized rats. SGP-T, at doses as low as 6.8×10^{-7}M, reduced antigen induced

contractions of these isolated intestinal tissues by 60% (data not shown).

Example 4: Modulation of Neutrophil Function by SGP-T

The subcutaneous implantation of a carrageenin-soaked sponge in rats is a model used to evaluate agents that modulate neutrophil chemotaxis, as carrageenin is a potent chemotactic agent and the sponge serves as a collecting reservoir.

Under halothane anaesthesia the sponge was removed from its subcutaneous site and the fluid was squeezed from it into a test tube. Following centrifugation at 2,000g for 10 min, the exudate was decanted and the remaining cells were suspended in 4 ml of 0.9% saline, and their number determined using a Coulter Counter. One ml of the remaining cell suspension was extracted for use in the superoxide assay.

Superoxide Assay

Neutrophils (10^6) were suspended in PBS buffer of the following composition: NaCl 137mM, KCl 2.7mM, Na_2HPO_4, 8.1mM, KH_2PO_4 1.47mM, $CaCl_2$ 1.19mM, $MgCl_2$ 0.54mM, glucose 7.5mM and cytochrome C 1.5mM (Sigma Chemical Co. St. Louis, MI) incubated at 37 C. Each sample was read along with a reference sample containing 1440 units of superoxide dismutase (Sigma) in a spectrophotometer (Hitachi, U200 spectrophotometer). The rate of superoxide production in response to 10^{-7}M phorbol myristate acetate (PMA) or 10^{-5} M N-formyl-methionyl-leucyl-phenylalanine (fMLP) was then inferred from the slope. (Derian et al., (1996), Biochemistry 35(4) : 1265-9).

Intravenous injections of SGP-T inhibited neutrophil influx into a carrageenin -soaked sponge in a dose-dependent manner (Figure 4). When the ability of neutrophils obtained from the carrageenin-soaked sponges to generate superoxide anion was evaluated, those obtained from saline-treated rats were totally refractory to *f*MLP and phorbol myristate acetate (PMA). In contrast, neutrophils collected from rats that received

intravenous (via the penile vein) SGP-T 4 hr. before implantation of the sponge were able to generate substantial amounts of superoxide (Figure 5).

Although the reasons for the lack of a superoxide response in carrageenin exposed neutrophils are unknown, receptor desensitization or uncoupling of the NADPH complex that generates the superoxide are possible explanations. SGP-T abrogates this desensitisation phenomena. By attenuating neutrophil chemotaxis, and by conserving the oxidative capacity of neutrophils, SGP-T provides a new anti-inflammatory agent. Treatment with SGP-T would limit an excessive movement of neutrophils into an inflammatory site, prevent an excessive and intensive generation of superoxide, but still allow the neutrophils to exert oxidative capacity essential for their fight against inflammatory stimuli.

Example 5: Effect of SGP-T on superoxide production of neutrophils

Neutrophils obtained either from carrageenin-soaked sponges implanted subcutaneously in rats, or from the blood of healthy human volunteers, were preincubated with various doses of SGP-T for 30 minutes, then stimulated with 10^{-7}M PMA and the rate of superoxide anion production determined. At doses less than 1μM, SGP-T inhibited superoxide anion production by both rat and human neutrophils, although the rat neutrophils were approximately 10-fold less sensitive than human neutrophils to this inhibitor effect (Figure 6). At higher doses of the peptide(> 1μM), an enhancement of superoxide anion production was evident with human neutrophils.

At 0.001 and 0.01 μM SGP-T, O_2^- production was inhibited by 15 to 20% in human neutrophils, while 10-fold higher concentrations were required for such inhibition with rat neutrophils. Much higher concentrations of SGP-T (10 and 20μM) stimulated O_2^- production by human neutrophils. Each value is presented

as the mean ± SEM, and the number of experiments was between 6 and 12.

Example 6: Structure-Activity Relationships

Analogues and fragments of SGP-T were tested for their ability to inhibit antigen (egg albumin)-induced intestinal contractions, using isolated jejunal segments from egg albumin-sensitised rats, as described in Example 3. Table 2 shows the analogues and fragments tested and the results obtained.

Example 7: Structure-Activity Relationships

Methods:

Animals and Tissue Preparation: Male Sprague-Dawley rats were sensitized to egg albumin (ovalbumin, OA) with 50ng of *Pertussis bordetella* toxin as an adjuvant. Four to six weeks after sensitization, the rats were anaethetised with sodium pentobarbital and the jejunum was removed. Two cm sections of the jejunum were mounted in tissue baths under 0.75 g of isometric tension, and the tissues were allowed to equilibrate for 20 min prior to beginning the experiment.

10µg of SGP-T or one of its analogues or fragments was added to a bath, while control tissues received the saline vehicle. Following a 10 min incubation, 100 ng of OA prepared in 0.9% saline was added. After the tissue had reached a maximal contractile response, the tissues were washed extensively to remove OA and peptide and, after the tissues had established baseline tension, 20µg of urecholine (URE; a cholinergic-muscarinic receptor agonist) was added to determine the maximal contractile response of the tissue. The tissues were then removed from the bath, cleared of all underlying mucosa and the weight of the remaining muscle was determined.

Peptides: A series of analogues or fragments of SGP-T was synthesized by conventional methods at the Protein Synthesis Facilities at the University of Calgary and Queen's University.

Data Analysis: The tension (measured in grams) per

gram tissue weight generated by each tissue in response to OA and URE was calculated. To normalize for differences in the ability of each tissue segment to contract in response to the muscle stimulants, the contractile response to URE was divided by that obtained upon addition of the sensitizing antigen, OA. Thus, a measure of the antigen-induced contraction was expressed as the URE/OA ratio. An increase in this ratio indicated that the contractile response to OA was decreased by the addition of a test substance.

The data was analyzed using the one-way analysis of variance (ANOVA) and differences between groups was determined using the Student t-test for unpaired samples. The results are shown in Figures 7 and 8 and Tables 3 and 4.

Structure Activity Relationships: Ovalbumin-sensitized jejunal tissues responded to the antigen (OA) with a contractile response, and the URE/OA ratio was 4.0 $\pm$ 0.4 (Table 3 and Figure 7; CON). This ratio indicates that URE elicited a contractile response that was 4 times larger than that induced by OA. The URE/OA ratio for SGP-T was 9.4 $\pm$ 1.3, indicating that 10 µg of this peptide reduced the anaphylactic reaction by more than 50%.

As examples of possible substitutions, various amino acids of SGP-T were individually replaced by alanine and the effect of these analogues was examined (Table 3 And Figure 7). Substitution of Aspartic acid (analogue A2) or Isoleucine (I: analogue A3) had a minimal effect on prevention of anaphylaxis. In contrast, the amino terminal Threonine (T; analogue A1), Phenylalanine (F; analogue A4) and to a lesser extent Glutamic acid (E; analogue A5) were important for the prevention of anaphylaxis. Removal of the two carboxy-terminal Glycines (analogue X6) or amidation of the terminal glycine (analogue X7) abolished anti-anaphylactic activity. In contrast, removal of the amino-terminal Threonine, Aspartic and Isoleucine

(analogue X8) had no effect on activity.

In view of the activity of the peptide FEGG, analogues of this peptide were examined in the same jejunal assay system (Table 4 and Figure 8).

In this series of experiments, the URE/OA ratio for control tissues was 5.7 ± 1.0 (Figure 8 and Table 4). The tripeptide FEG, which contains 2 fewer amino acids than FEGGG, attenuates antigen-induced contraction of the sensitized jejunal segments to the same degree as intact SGP-T. The carboxy-terminal Glycine was replaced with a sarcosine which places a methylene (CH_3) group on the alpha carbon of the glycine molecule, without a significant change in biological activity.

Substitution of glycine with a large basic amino acid (Citrulline; Cit), however, or conformationally restricting the carboxy-terminal by substituting a Proline (Pro) for the Glycine, resulted in loss of biological activity.

Example 8: Effects of Analogues of SGP-T on Anaphylactic Hypotension

Methods:

Animals and Tissue Preparation: Male Sprague-Dawley rats were sensitized to egg albumin (ovalbumin; OA) with 50 ng of *Pertussin bortella* toxin as an adjuvant. Four to six weeks after sensitization, the rats were anesthetized with sodium pentobarbital. A tracheal tube was inserted and a cannula was inserted into the left ventricle for measuring ventricular peak systolic pressure (VPSP). This cannula was coupled to a blood pressure transducer. A cannula was also inserted into the jugular view for administration of peptide (100µg/kg) and antigen (100 µg of OA). The peptides were injected intravenously 10 min prior to antigen injection and blood pressure was recorded for 30 min.

In a second study, the peptide feG (D-phenylalanine, D-glutamate, glycine) was administered orally by a stomach tube 1 hour prior to intravenous injection of the

sensitizing antigen.

Data Analysis: The percent changes in VSPS induced by anaphyalctic reaction were calculated relative to baseline VPSPs. Significance differences within groups were determined with one-way analysis of variance (ANOVA), and between groups with the Student t-test.

The intravenous administration of SGP-T, FEG and feG, at doses of 100 µg/kg, significantly inhibited anaphylactic hypotension, as seen in Figure 9. feG also significantly reduced anaphylactic hypotension when administered orally, as seen in Figure 10.

Example 9: Effect of Analogues of SGP-T on Anaphylactic Perturbation of Intestinal Motility

Methods:

Animals and Tissue Preparation: Male Hooded-Listar rats were sensitized to egg albumin (ovalbumin; OA). Two weeks after sensitization, the animals had electrodes implanted into the muscle of the jejunum for recording muscle electrical activity. One week later, the fasted rats received SGP-T or an analogue, either intravenously (injection via penile vein under halothane anaesthesia) or orally (via gastric tube), 20 min before challenge with OA, or bovine serum albumin as control, by intragastric administration.

The disruption of intestinal migrating myoelectric complexes (MMCs) (Scott et al., (1988), J. Physiol., v. 255, pp. G505-511), a measure of intestinal activity, and the presence of diarrhea were used as biological measurements of anaphylaxis. The results are expressed as the percent of the rats exhibiting disrupted MMCs and diarrhea.

Control rats which received an intravenous injection of vehicle (0.9% saline) showed anaphylactic reactions when the sensitizing antigen, OA, was administered intragastrically (Figure 11). One hundred percent of the rats had disrupted MMC's and 90% exhibited the clinical signal of anaphylaxis, diarrhea. The non-sensitizing antigen, BSA, did not elicit an anaphylactic reaction.

SGP-T, FEG and feG (each administered intravenously at 100 µg/kg) prevented or reduced intestinal anaphylaxis induced by OA. Oral treatment with 350 µg/kg of feG totally abolished all signs of anaphylaxis, as none of the eight animals tested exhibited either diarrhea or disruption of intestinal motility (Figure 11).

These studies confirm that the tripeptide, FEG, a carboxy terminal fragment of SGP-T, is sufficient to inhibit intestinal anaphyalctic reactions, and that a metabolically stable form of this tripeptide, feG, also possesses anti-anaphylactic activity. These three peptides may be useful in reducing severity of anaphylactic reactions in sensitized individuals.

Example 10

Methods: Sprague-Dawley rats (200-300g) were sensitized with 1 mg ovalbumin and 50 ng *Pertussin toxin* (Sigma) (24) one month prior to experimentation. The submandibular glands were removed bilaterally 7 days prior to the experiment by previously described procedures (23). Rats were anesthetized with sodium pentobarbital (65 mg/kg) and a tracheal tube (PE240 polyethylene tubing) was inserted before performing further surgeries. The jugular vein was cannulated for injection of ovalbumin antigen, which was given as a bolus (100 µg/kg) over 10 sec. The right carotid artery was cannulated and the cannula tip was slowly advanced into the left ventricle, as detected when diastolic pressure fell to near 0 mmHg. The ventricular cannula was connected to a TXD-310 transducer and heart function parameters were recorded using a Digi-Med Heart Performance Analyser (Micro-Med, Louisville, KY). The effects of anaphylaxis on heart function were analysed by comparing the changes in ventricular peak systolic pressure (VPSP), and in the rate of ventricular contraction during systole (dP/dt) and relaxation during distole (-dP/dt). Antigen was injected at 10 min. The anaphylactic response was followed for 30 min.

Very rapidly (within 1 min) after the intravenous injection of antigen into ovalbumin-sensitized rats, VPSP dropped and minimal ventricular pressures were measured at 5 min after initiation of the anaphylactic reaction.

In control rats, minimal VPSP decreased by 36.5 ± 7.2% from the pre-antigen values of 165.3 ± 6.3 mm Hg. Over the next 25 min, blood pressure recovered slowly such that, at 30 min after antigen injection, VPSP was only 11.5 ± 4.4% lower than the pre-antigen VPSP values.

Submandibular gland peptide, SGP-T, when injected 10 min prior to the antigen, protected the rats against anaphylactic hypotension (Fig. 12). The average decrease in VPSP over the 30 min of the experiment in control rats (saline pretreatment) was 46.2 ± 6.5 mm Hg, whereas in rats that received 10 and 100 µg/kg of SGP-T, the integrated decreases in VPSP were only 16.7 ± 8.3 and 15.9 ± 8.0 mm Hg, respectively. By the end of the experiment, VPSP had attained pre-antigen values in these SGP-T treated rats. Neither lower (10 µg/kg) nor higher doses (350 µg/kg) of SGP-T protected against antigen elicited hypotension. The end diastolic pressure (EDP) was not significantly affected by the anaphylactic response.

To determine if anaphylaxis directly affected heart function, the percent changes in VPSP were plotted against percent changes in dP/dt and -dP/dt. No differences were noted in the slopes of the regression lines of dP/dt or -dP/dt (Table 5) between rats receiving saline and those pretreated with either 35 or 100 µg/kg SGP-T. The regression coefficients were all above 0.9. By analysis of variance, the slopes of the regression lines of dP/dt and -dP/dt were not different from unity.

The rats were anesthetized with intraperitoneal sodium pentobarbital (65 mg/kg), a tracheotomy was performed and the right jugular vein was cannulted (PE50 tubing) for administration of endotoxin. The right carotid artery was cannulated (PE50 tubing) and the

cannula tip was slowly advanced into the left ventricle, as detected when diastolic pressure fell to near 0 mmHg. The ventricular cannula was connected to a TXD-310 transducer and heart function parameters were recorded using a Digi-Med Heart Performance Analyzer, Model 200, (Micro-Med, Louisville, KY). The data was captured using Digi-med System Integrator, Model 200 (Micro-Med).

Following a 15 to 20 min stabilization period endotoxin (3.5 mg/kg of LPS from Salmonella typhosa; Sigma Chemical Co. St. Louis, MO) was injected slowly over 1 min via the jugular vein, and heart function variables were monitored at 1 min intervals for 60 min by sampling the last 10 sec of every minute. The effect of endotoxin on ventricular function variables was evaluated by assessing absolute values of the variables, and their percent changes relative to pre-endotoxin values. The average absolute and percent changes were calculated over the 60 min of the experiment (1-60 min) and at four time intervals (1-15 min, 16-30 min, 31-45 min and 46-60 min) following endotoxin injection. All data are expressed as mean ± SEM. Statistical significance was determined using the Student t-test to identify differences between groups.

The results are shown in Figure 13. SGP-T reduced significantly the decrease in ventricular peak systolic pressure (VPSP) provoked by antigen (100 µg/kg) administration to ovalbumin-sensitized rats.

A reduction in the severity of decrease in VPSP shows that SGP-T at doses of 35 and 100 µg/kg significantly reduced the drop in blood pressure elicited by the anaphylactic reaction.

Example 11

The effect of the tripeptide FEG on endotoxin-induced hypotension was examined, using the methods described in Example 10. 100 µg/kg FEG was given iv. 30 mins before injection of 3.5 mg/kg LPS (Salmonella typhosa). Examination of the mean arterial blood

pressure showed that FEG did not influence endotoxin-induced hypotension, as seen in Figure 14, which shows MABP at 10 min prior to LPS injection (Before) and 60 min after LPS injection (After).

Example 12

Rats were operated on to remove salivary glands (or controls were sham-operated) one week before challenge with endotoxin. At the time of operation, a temperature sensitive radio transmitter was inserted in the peritoneal cavity. One week after operation, baseline temperatures were recorded by telemetry and SGP-T or saline was injected immediately before intra-peritoneal injection of 150 µg/kg LPS. Temperature was followed for 12 hours. Results are shown in Figure 15. SGP-T suppressed a late phase (180 - 420 minutes) of endotoxin-induced fever.

The present invention is not limited to the features of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

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TABLE 1

Unoperated Rats

Treatment	MABPbef	MABPaft	decMABP	%Dec
Saline (18)	122.3±3.9	88.0±3.6	-34.2±3.8	-27.6±2.5
SGP-T (18)	113.2±3.8	99.6+3.8*	-13.6+2.9**	-11.6+2.4**
SGP-S (15)	123.4±3.0	95.2±4.3	-28.1±1.8*	-22.9±1.3

Sialadenectomized Rats

Treatment	MABPbef	MABPaft	decMABP	%Dec
Saline (22)	125.0±3.8	68.0±3.4	-57.3±4.6	-44.7±3.1
SGP-T (18)	123.3±4.9	77.2±3.9	-46.1±4.9	-36.3±3.3
SGP-S (20)	119.7±1.8	81.6+5.8*	-37.6+4.4*	-32.3+3.9*

MABPbef = mean arterial blood pressure before LPS; MABPaft = average MABP for 60 mins after LPS injection; dec MABP = decrease in MABP after LPS relative to MABPbef; %Dec = percent decrease in MABP relative to MABPbef. *different from Saline; **different from Saline and SGP-S or SGP-T.

TABLE 2

Identification Number	Peptide	Sequence ID No.	Biological Activity
T2:	STDIFEGG	10	-
SGP-T:	TDIFEGG	8	+++
T3:	ADIFEGG	2	-
T7:	TAIFEGG	3	+++
T10:	TDAFEGG	4	+
T11:	TDIAEGG	5	-
T9:	TDIFAGG	6	-
T5:	TDIFEGG-NH_2	8	
T4:	TDIFE	7	-
T6:	FEGGG	9	++
T8:	FEG		++

Biological activity = inhibition of antigen-induced jejunal contraction by test peptide. +++ = highest inhibition (~ 60%), ++ = moderate inhibition (~ 40%), + = lowest inhibition (~ 20%), - = no inhibition.

TABLE 3

Test Substance	Amino acid Sequence	URE/OA
Saline		4.0 ± 0.4
SGP-T	TDIFEGG	9.4 ± 1.3 *
A1	ADIFEGG	3.6 ± 0.9
A2	TAIFEGG	12.3 ± 2.2 *
A3	TDAFEGG	8.3 ± 1.9 *
A4	TDIAEGG	5.2 ± 1.2
A5	TDIFAGG	7.2 ± 1.1 *
X6	TDIFE	1.7 ± 0.3
X7	TDIFEGG-NH_2	4.3 ± 0.7
X8	FEGGG	10.7 ± 2.0 *
X9	STDIFEGG	3.58 ± 1.07

*P < 0.05

TABLE 4

Test Substance	Amino acid Sequence	URE/OA
Saline		5.7 ± 1.0
SGP-T	TDIFEGG	13.9 ± 5.6
FEG	FEG	12.1 ± 2.8
Sar	FE-Sarcosine	10.0 ± 1.6
Cit	FE-Citrulline	5.0 ± 1.1
Pro	FE-Proline	6.2 ± 1.3

TABLE 5

Linear relationship between % VPSP and % dP/dt and % -dP/dt.

Treatment	Equation of Regression Lines	dP/dt - dP/dt
Saline	$Y=1.19(\pm0.04)X+0.02(\pm0.02)$	$Y=1.39(\pm0.05)X+0.05(\pm0.03)$
35μg/kg SGP-T	$Y=1.17(\pm0.09)X-0.01(\pm0.01)$	$Y=1.62(\pm0.09)X+0.19(\pm0.03)$
10μg/kg SGP-T	$Y=1.00(\pm0.05)X+0.02(\pm0.02)$	$Y=1.55(\pm0.04)X+0.01(\pm0.02)$

We claim:

1. A peptide of the formula: $R^1 - X^1 - X^2 - R^2$
wherein X^1 is an aromatic amino acid residue;
X^2 is any amino acid residue;
R^1 is NH_2 - or an amino acid sequence X^3 - X^4 - X^5
wherein X^3 is an aliphatic amino acid residue having a side chain hydroxyl group and X^4 and X^5 are the same or different and are any amino acid residue and wherein R^2 is a sequence of 1 to 3 amino acid residues which are the same or different and are aliphatic amino acid residues.

2. The peptide of claim 1
wherein X^1 is Phe;
X^2 is Glu or Ala;
R^2 is Gly-Gly;
R^1 is X^3- X^4- X^5 wherein
X^3 is Thr,
X^4 is Asp or Ala and
X^5 is Ile or Ala.

3. The peptide of claim 1
wherein R^1 is NH_2-;
X^1 is an aromatic amino acid;
X^2 is Glu or Ala and
R^2 is Gly, Gly-Gly, Gly-Gly-Gly or sarcosine.

4. The peptide of claim 3
wherein X^1 is Phe and X^2 is Glu.

5. The peptide of claim 2 having an amino acid sequence selected from the group
consisting of:
(a) Thr-Asp-Ile-Phe-Glu-Gly-Gly (Sequence ID NO:8);
(b) Thr-Ala-Ile-Phe-Glu-Gly-Gly (Sequence ID NO:3);
(c) Thr-Asp-Ala-Phe-Glu-Gly-Gly (Sequence ID NO:4); and

(d) Thr-Asp-Ile-Phe-Ala-Gly-Gly (Sequence ID NO:6).

6. The peptide of claim 3 having an amino acid sequence selected from the group consisting of:

(a) Phe-Glu-Gly-Gly-Gly (Sequence ID NO:9);

(b) Phe-Glu-Gly; and

(c) Phe-Glu-Sarcosine.

7. The peptide of claim 1 wherein R^2 is a sequence of 1 to 3 amino acid residues which are the same or different and are selected from the group consisting of glycine, sarcosine, azetidine, nipecotic acid and pipecotic acid.

8. The peptide of claim 3 wherein R^2 is a sequence of 1 to 3 amino acid residues which are the same or different and are selected from the group consisting of glycine, sarcosine, azetidine, nipecotic acid and pipecotic acid.

9. The peptide of any of claims 1 to 8 wherein at least one amino acid is a D amino acid.

10. The peptide of claim 4 or 6 wherein Phe and Glu are D amino acids.

11. A peptide having the amino acid sequence Ser-Gly-Glu-Gly-Val-Arg (Sequence ID NO:1).

12. A pharmaceutical composition comprising a peptide of any of claims 1 to 11 and a pharmaceutically acceptable carrier.

13. A method for treating or preventing SIRS-induced hypotension in a mammal comprising administering to the mammal an effective amount of a peptide of any of claims 1, 2, 5, 7, 9 or 11 or of an effective fragment or derivative of said peptide.

14. A method for treating or preventing anaphylactic

hypotension in a mammal comprising administering to the mammal an effective amount of a peptide of any of claims 1 to 10, or of an effective fragment or derivative of said peptide.

15. A method of reducing or preventing an anaphylactic reaction in a mammal comprising administering an effective amount of a peptide of any of claims 1 to 10 or of an effective fragment or derivative of said peptide to the mammal.

16. A method of reducing or preventing an endotoxic reaction in a mammal comprising administering an effective amount of a peptide of any of claims 1, 2, 5, 7, 9 or 11 or an effective fragment or derivative of said peptide to the mammal.

17. A method for treating an inflammatory disorder in a mammal comprising administering to the mammal an effective amount of a peptide of any claims 1, 2, 5, 7, 9 or 10 or of an effective fragment or derivative of the peptide to the mammal.

18. The method of claim 17 wherein the inflammatory disorder is selected from the group consisting of a rheumatic disorder, inflammatory bowel disease, post-ischemic inflammation or systemic inflammatory response syndrome.

19. An antibody which specifically recognises an epitope of a peptide of any of claims 1 to 11.

20. A method of determining the peptide SGP-T or the peptide SGPS in a biological fluid comprising obtaining a sample of the biological fluid and determining the peptide in the fluid by immunoassay employing an antibody of claim 19.

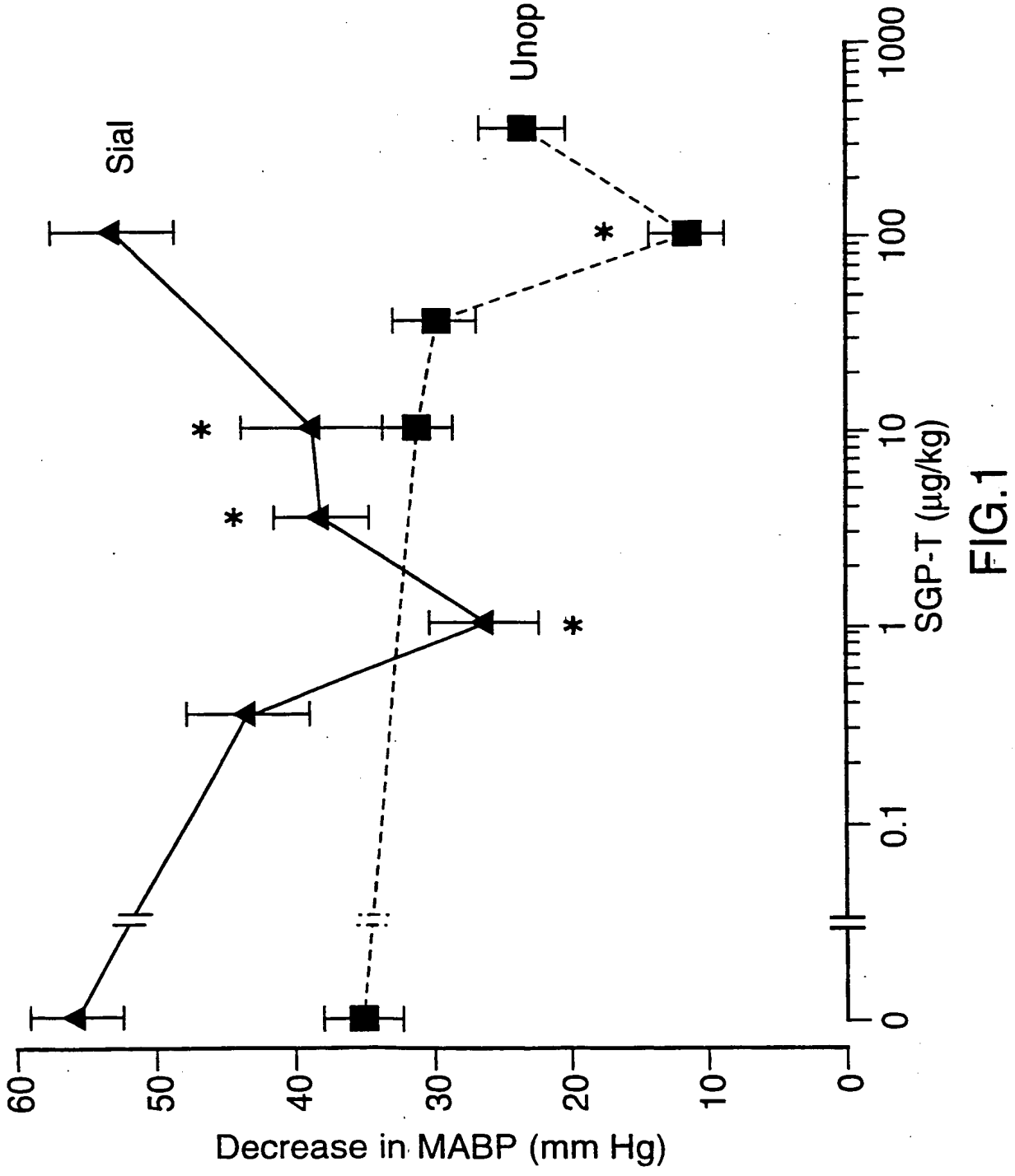
Unop
Sial
*
*
*
*
60
50
40
30
20
10
0
Decrease in MABP (mm Hg)
0
0.1
1
10
100
1000
SGP-T (µg/kg)

FIG.1

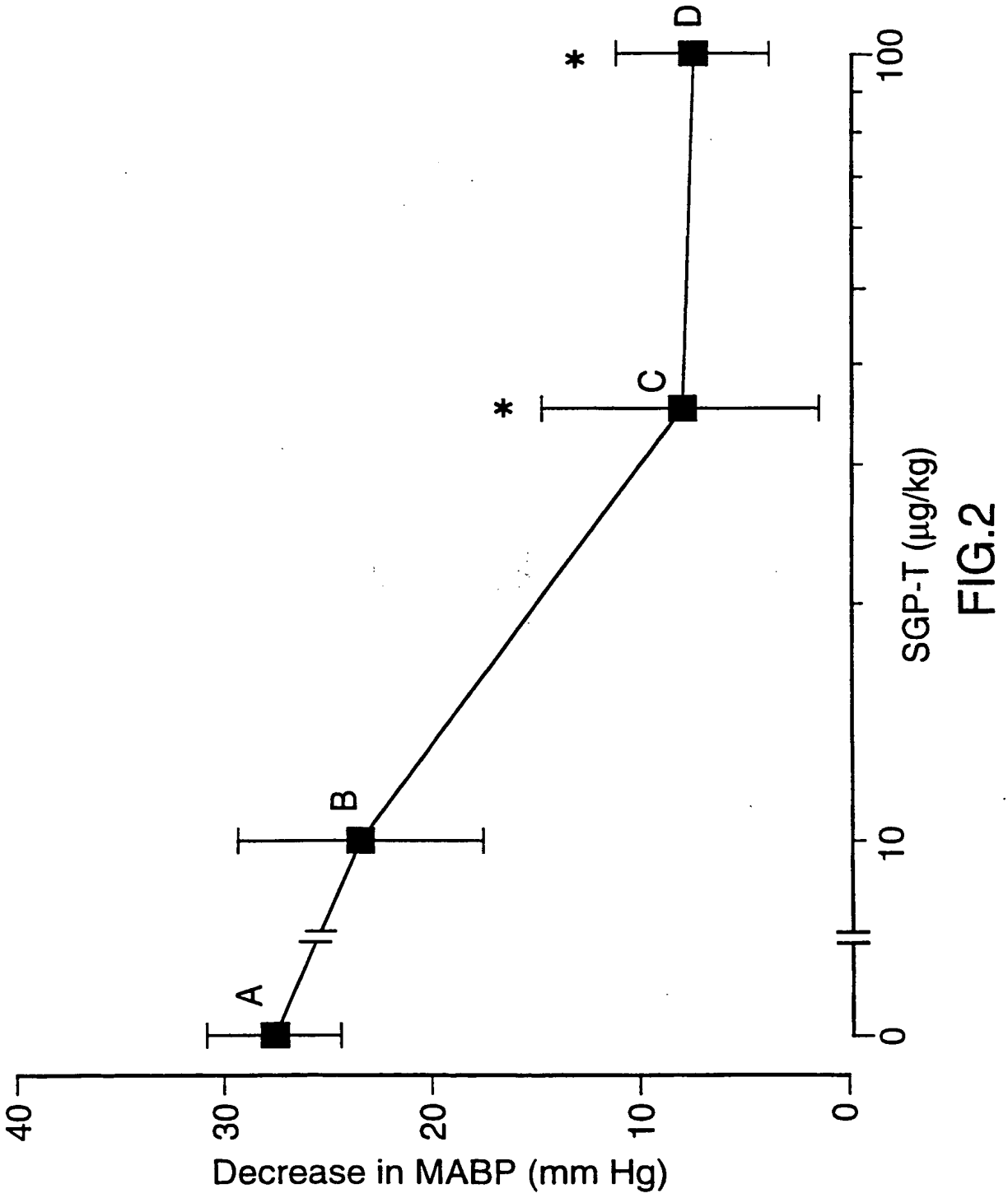

FIG.2

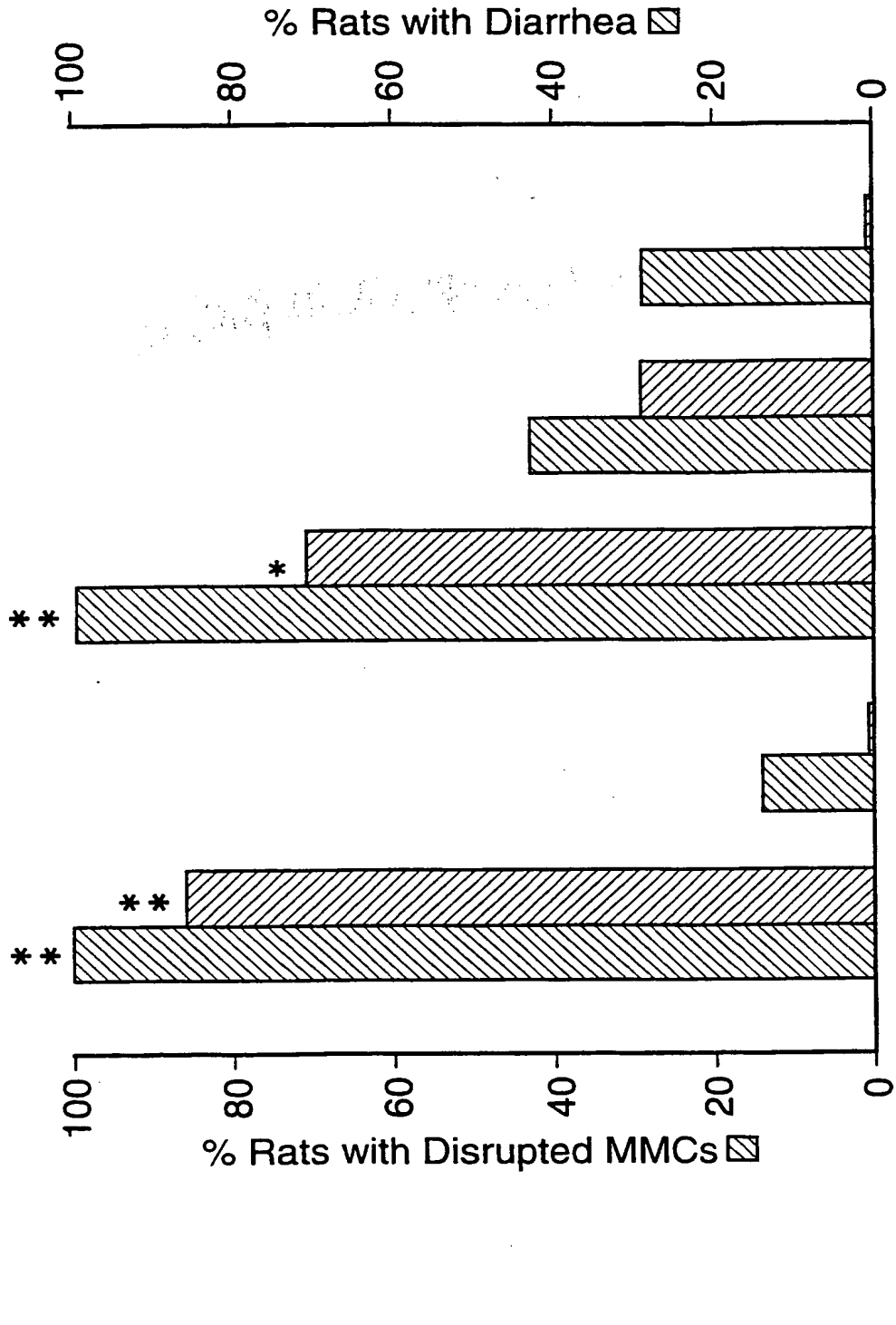
% Rats with Diarrhea
100
80
60
40
20
0
100ug
10ug
1ug
Saline
Saline
SGP-T (µg/kg)
EA
EA
EA
BSA
EA
PRETREATMENT
CHALLENGE
*
**
**
**
% Rats with Disrupted MMCs
100
80
60
40
20
0

FIG.3

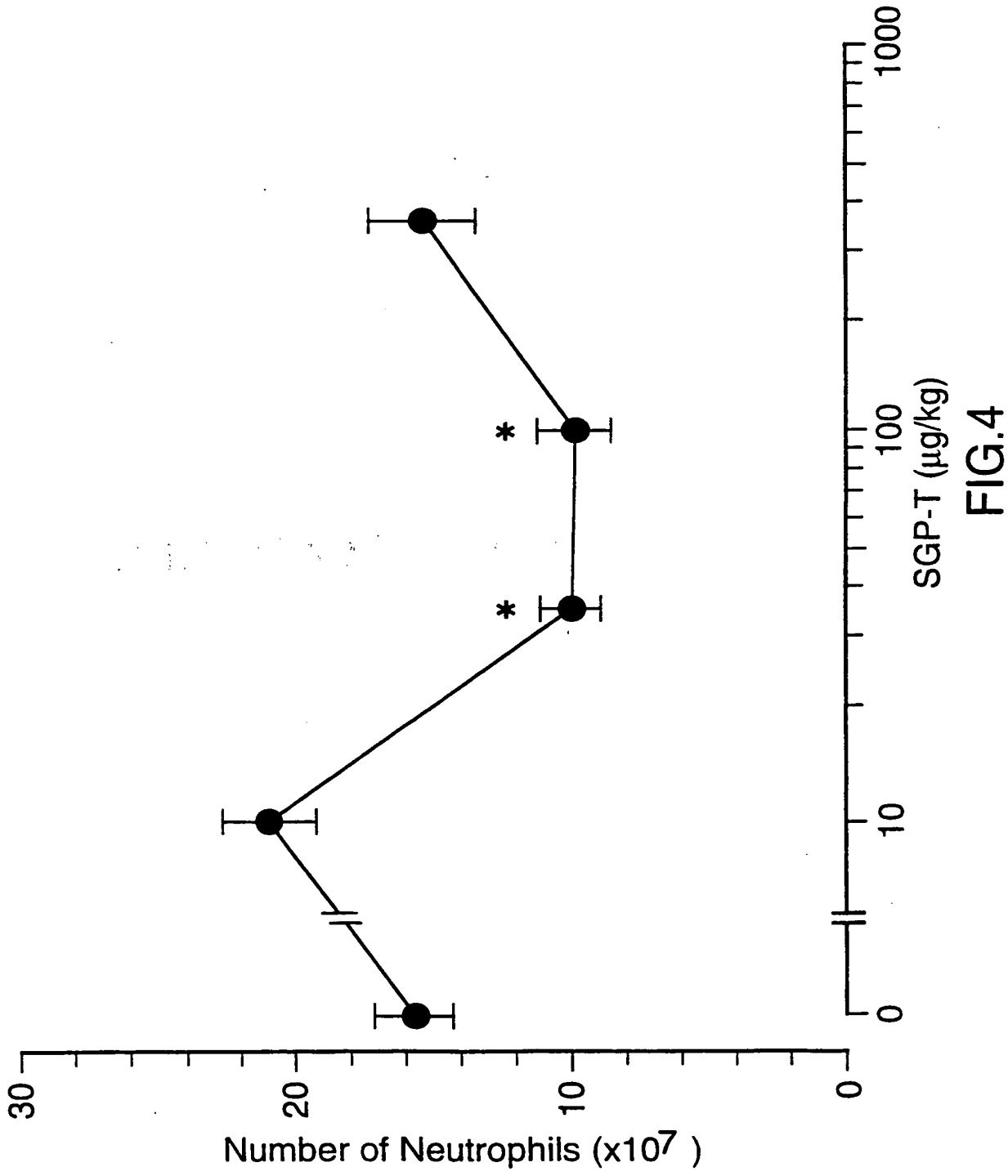

FIG.4

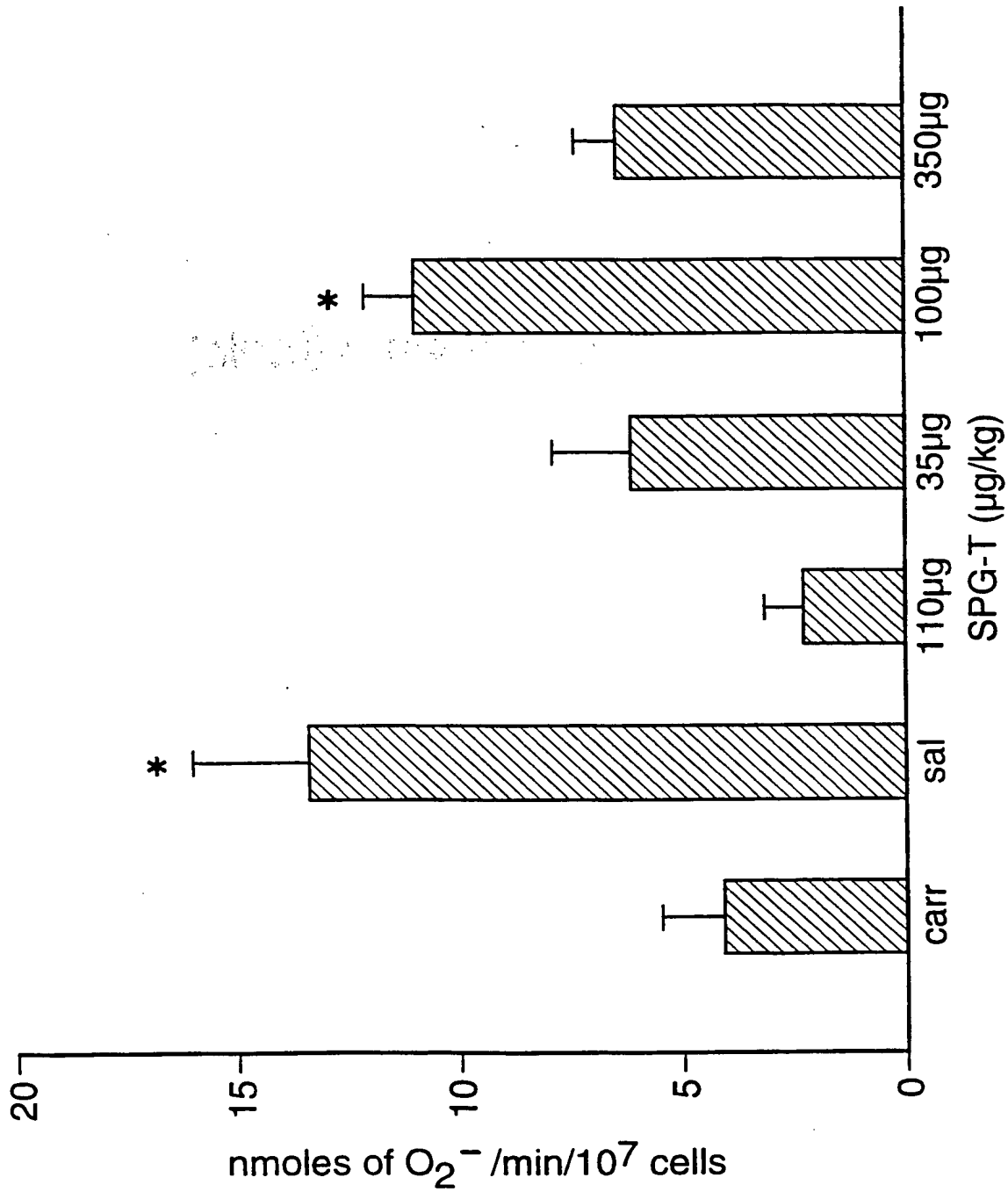
carr
sal
110µg
35µg
100µg
350µg
SPG-T (µg/kg)
*
*
0
5
10
15
20
nmoles of O_2^- /min/10^7 cells

FIG.5

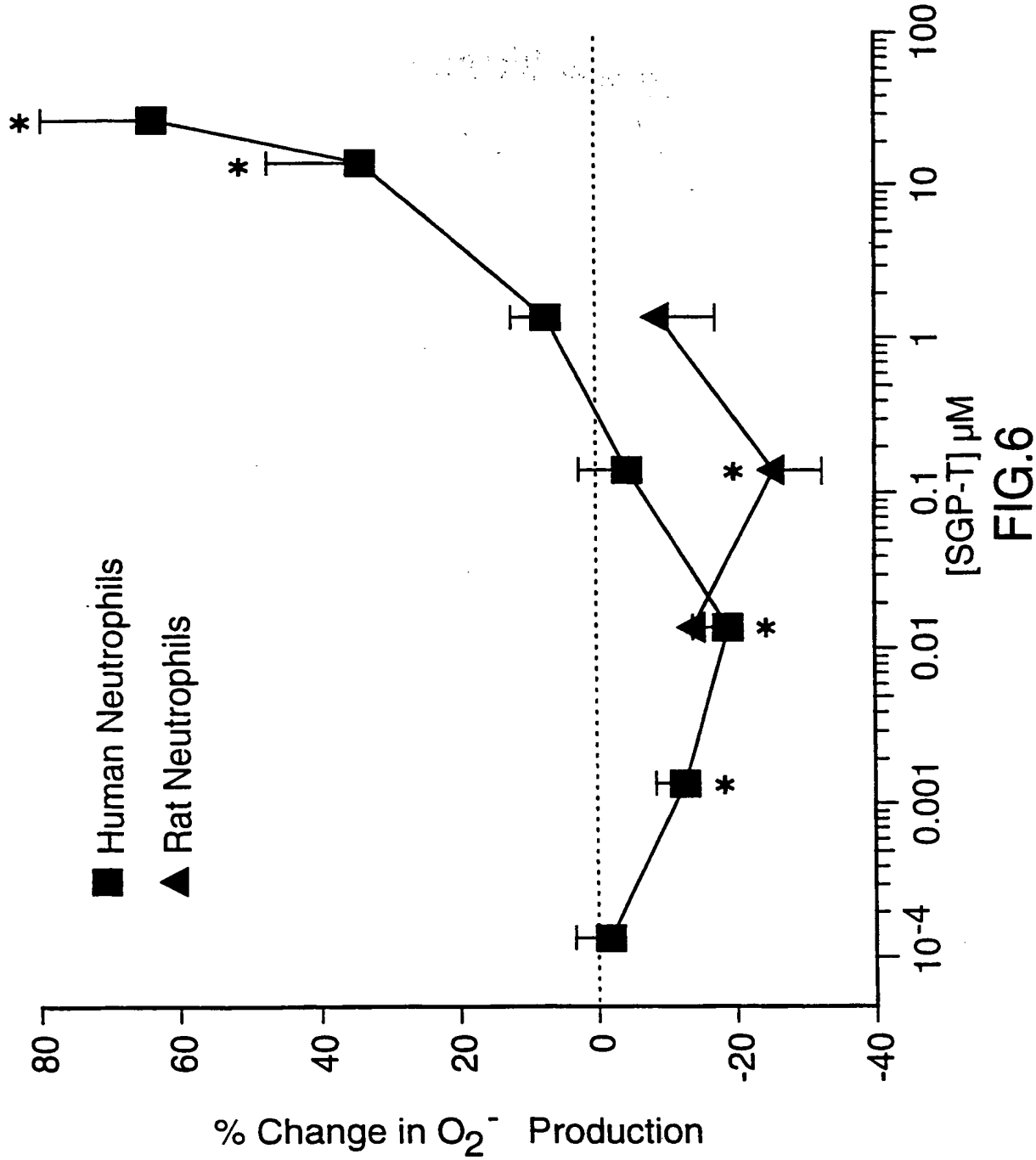
Human Neutrophils
Rat Neutrophils
% Change in O_2^- Production
80
60
40
20
0
-20
-40
10^{-4}
0.001
0.01
0.1
1
10
100
[SGP-T] µM
*

FIG.6

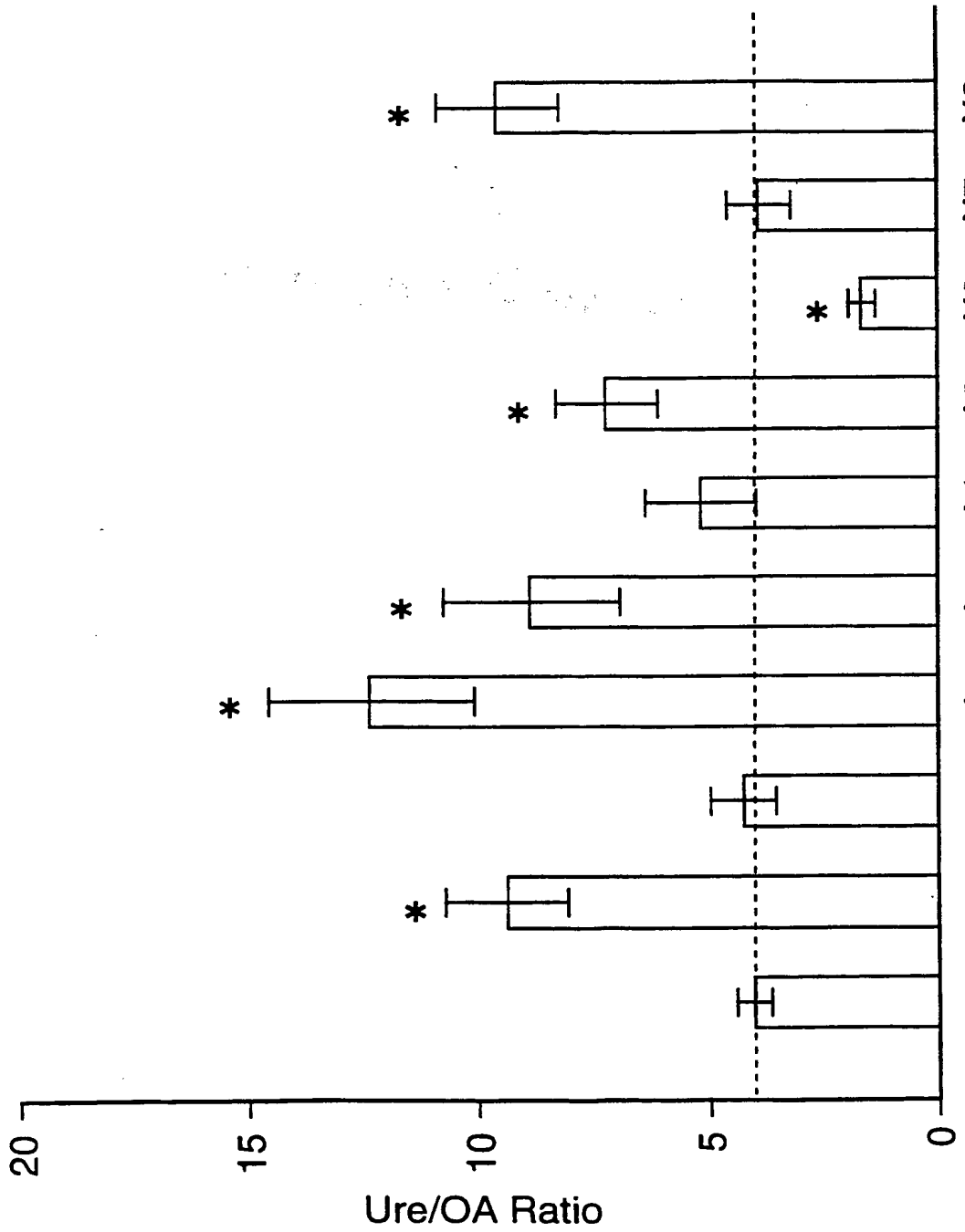

FIG.7

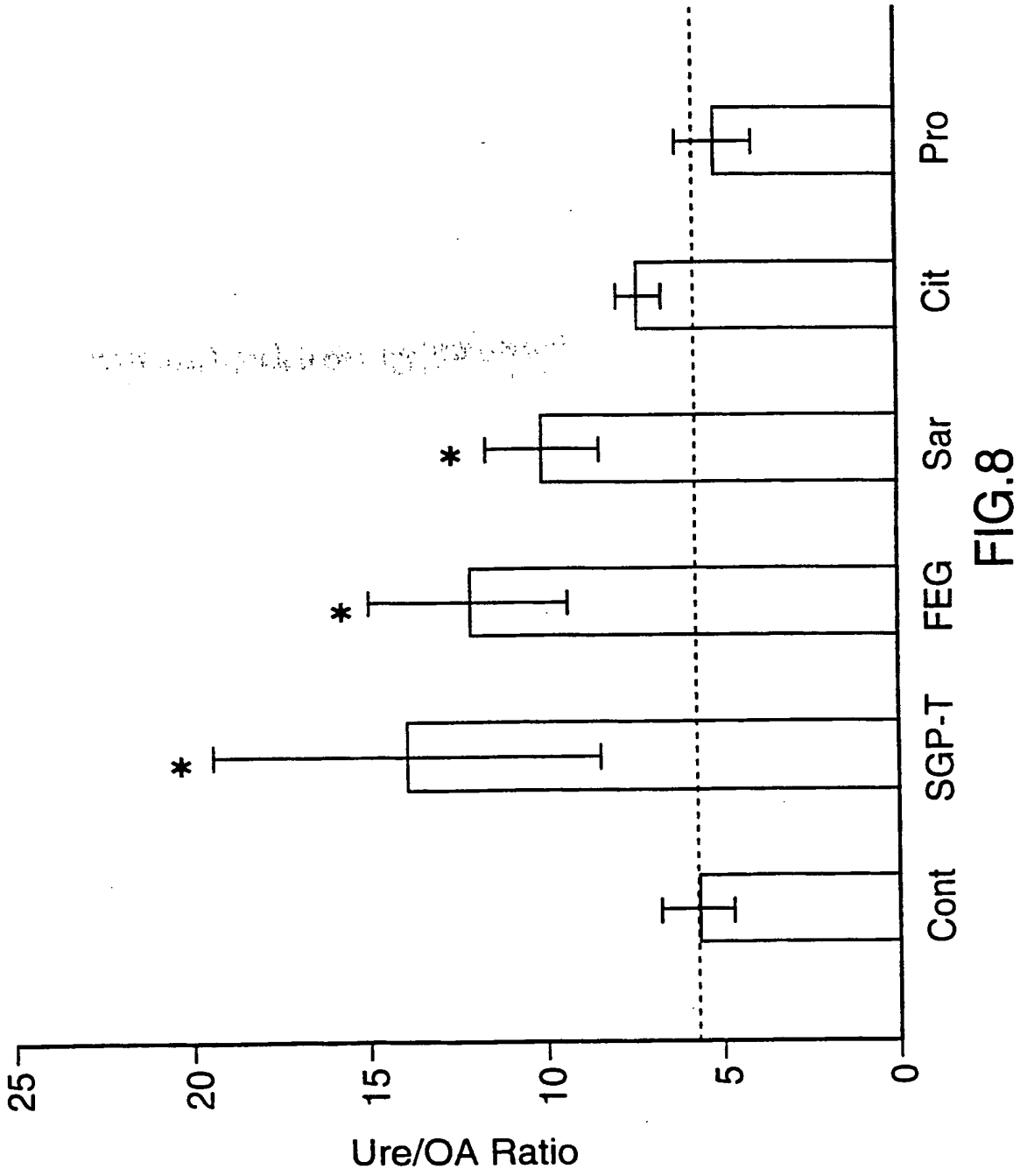

FIG.8

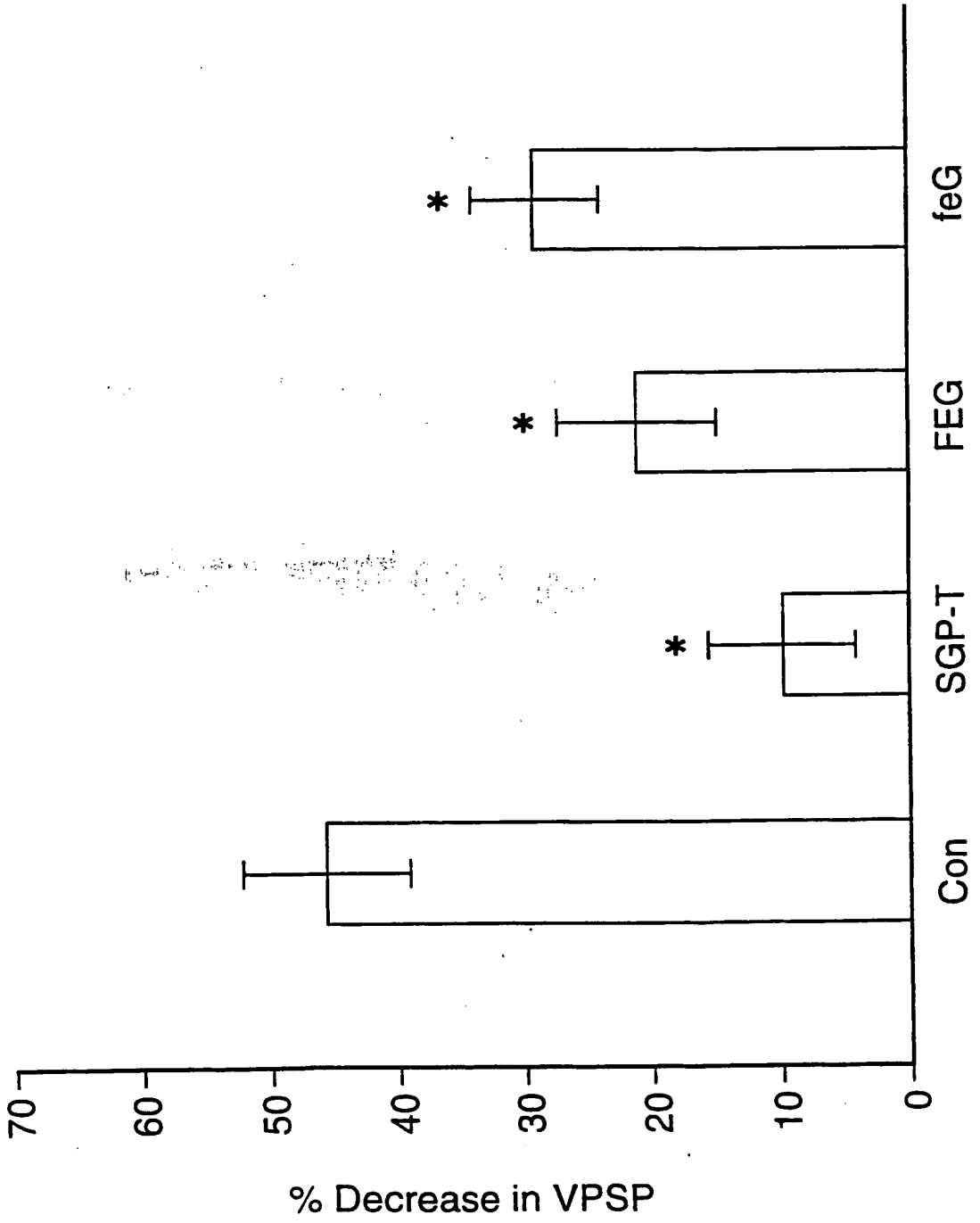

FIG.9

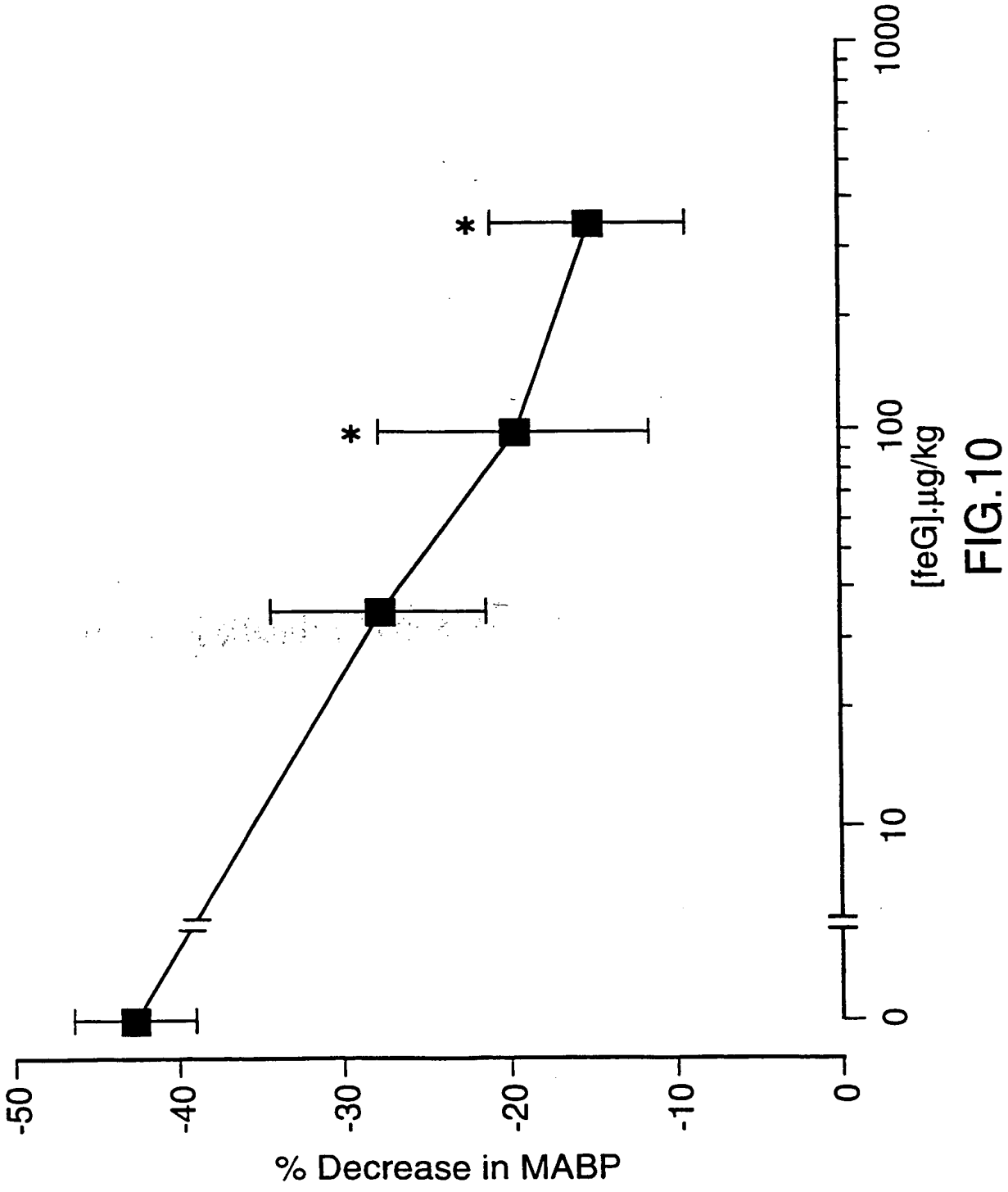

FIG.10

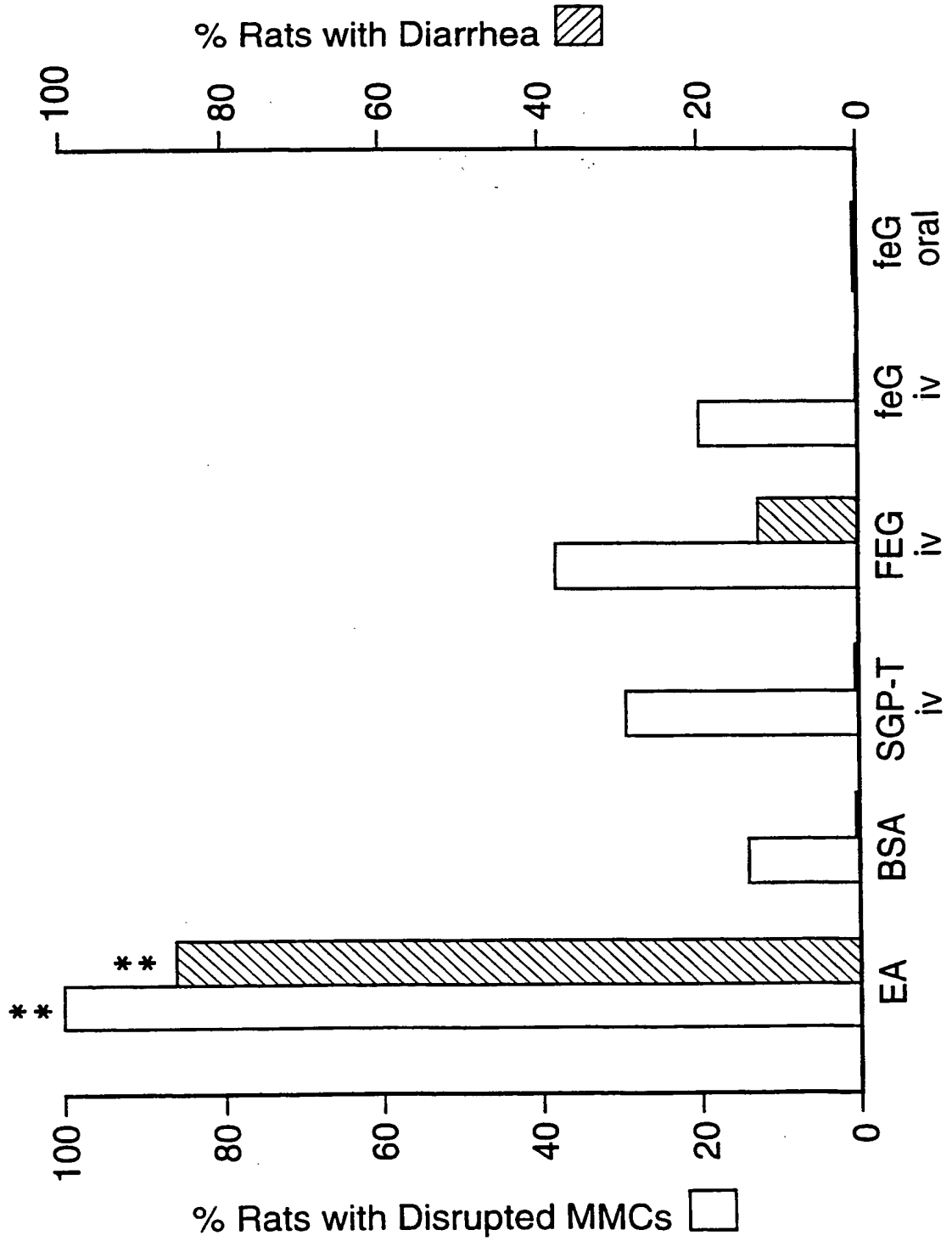
% Rats with Diarrhea
100
80
60
40
20
0
feG oral
feG iv
FEG iv
SGP-T iv
BSA
EA
**
**
100
80
60
40
20
0
% Rats with Disrupted MMCs

FIG.11

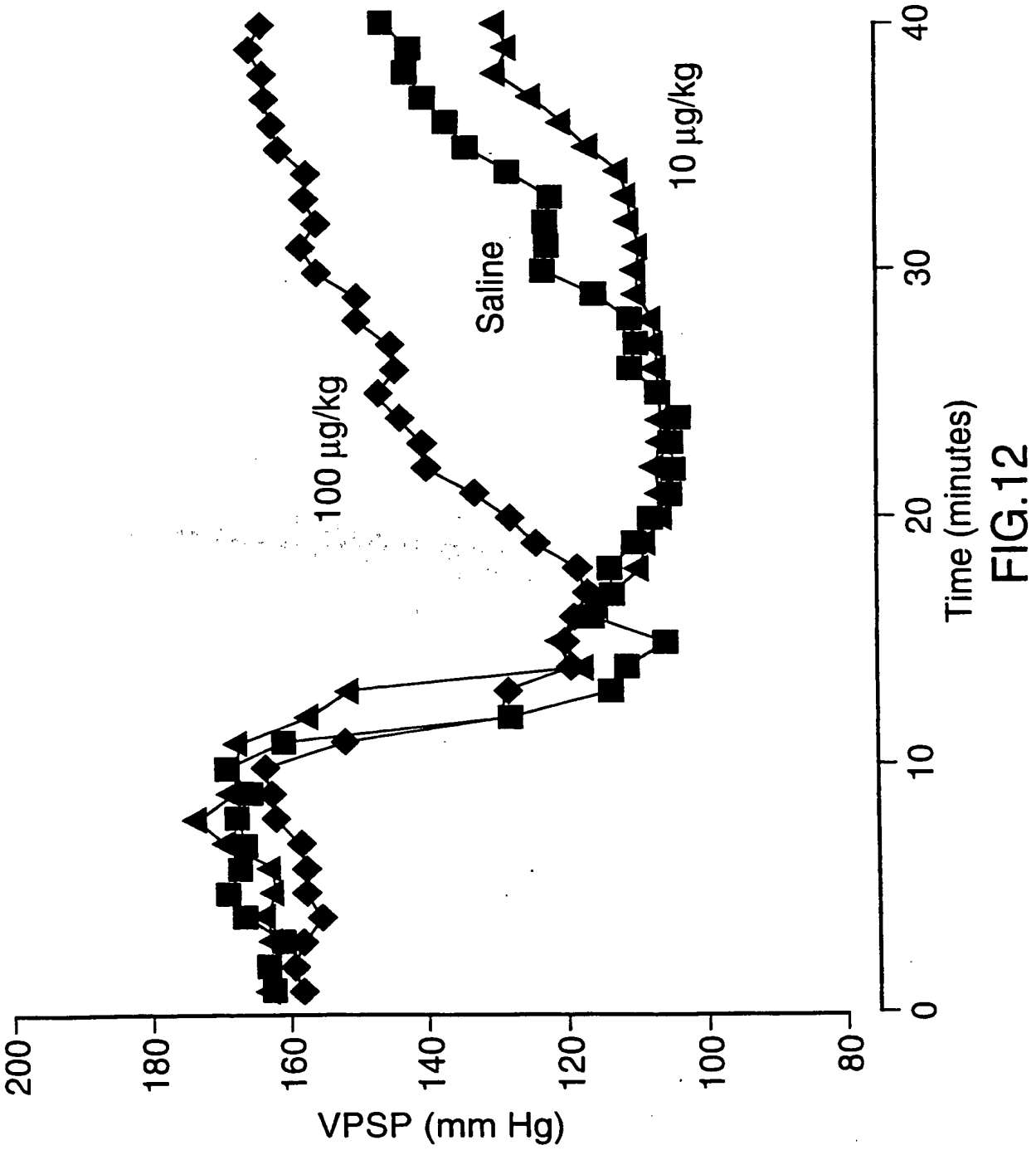
100 µg/kg
Saline
10 µg/kg
200
180
160
140
120
100
80
VPSP (mm Hg)
0
10
20
30
40
Time (minutes)

FIG.12

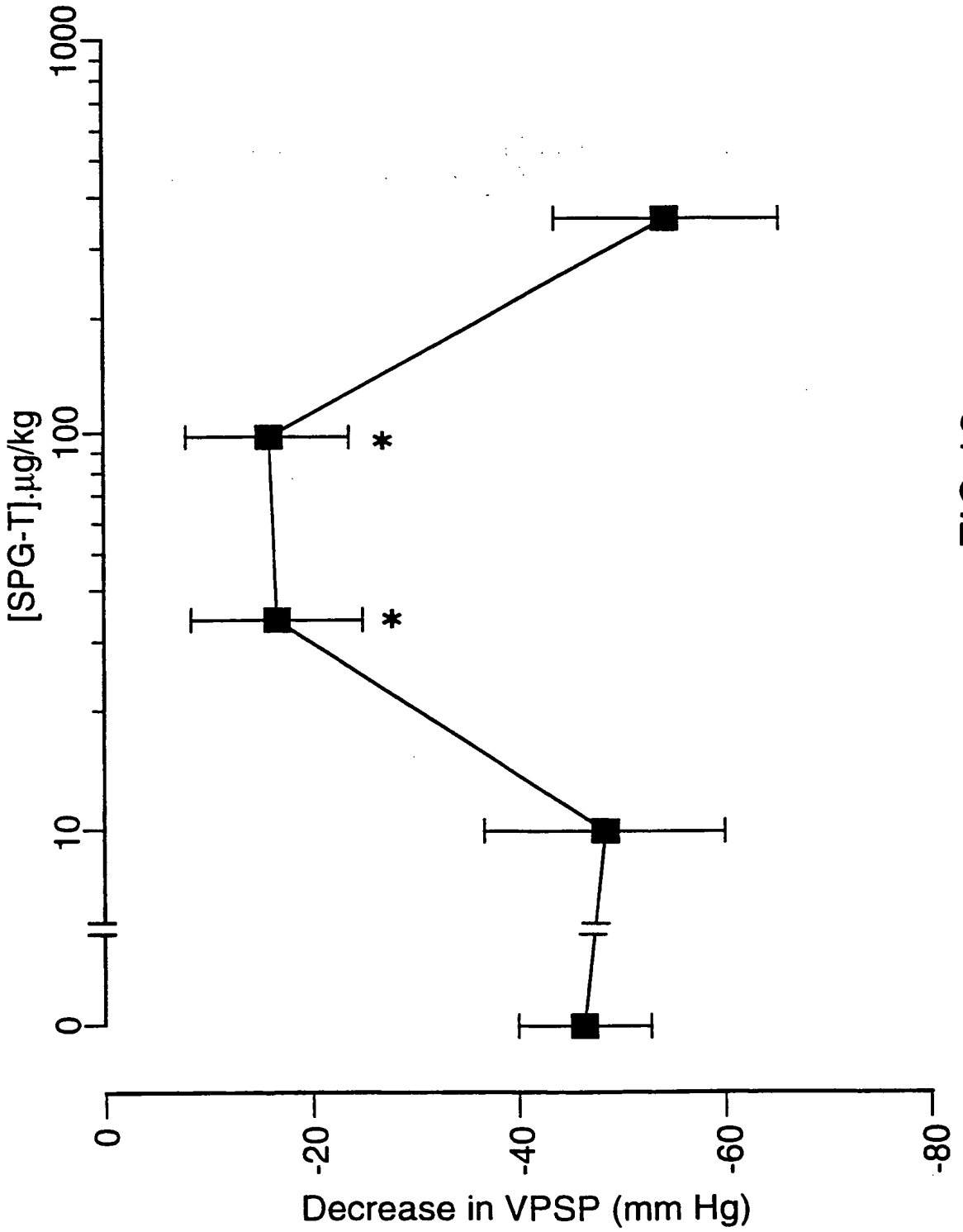

FIG.13

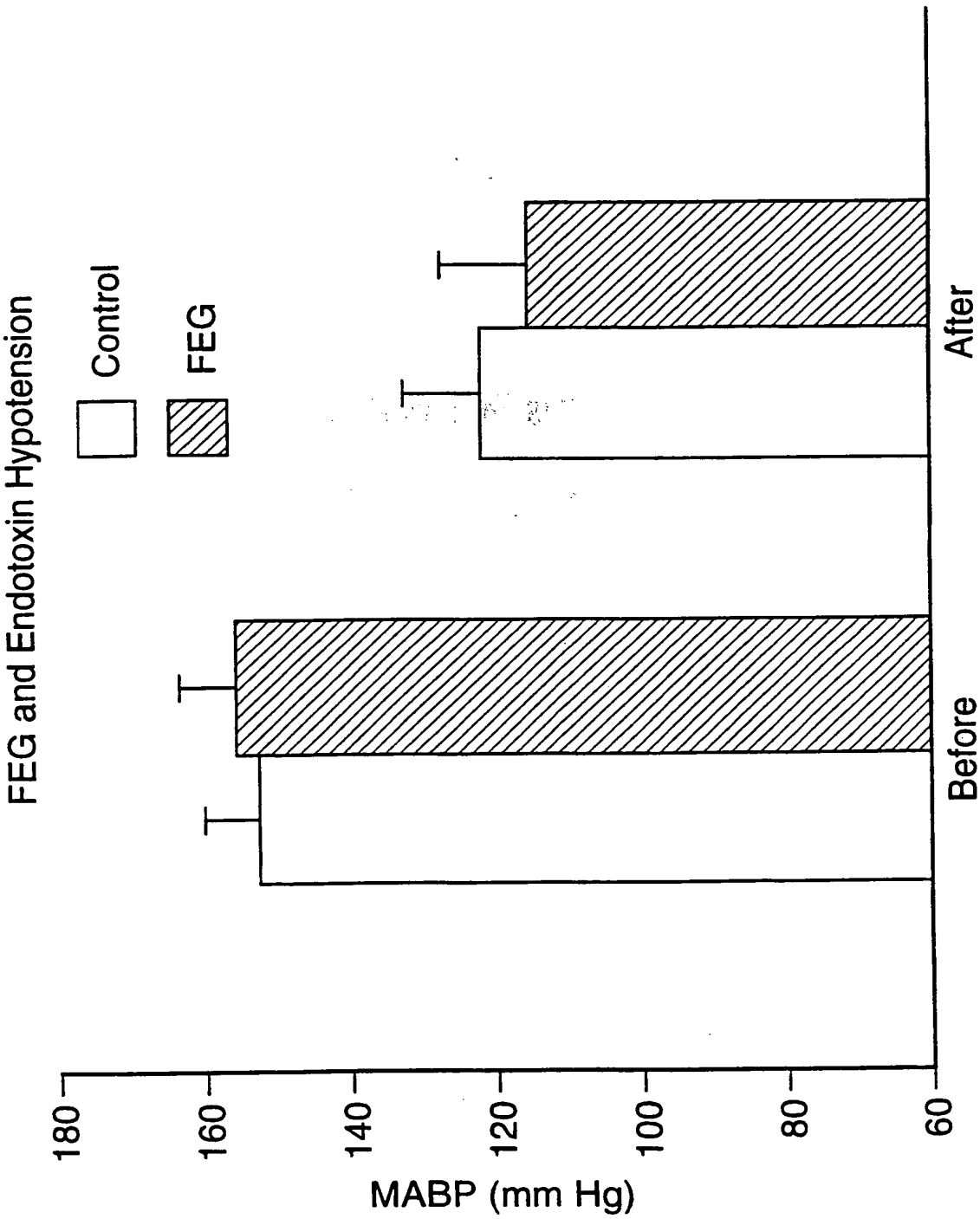
FEG and Endotoxin Hypotension
Control
FEG
After
Before
180
160
140
120
100
80
60
MABP (mm Hg)

FIG.14

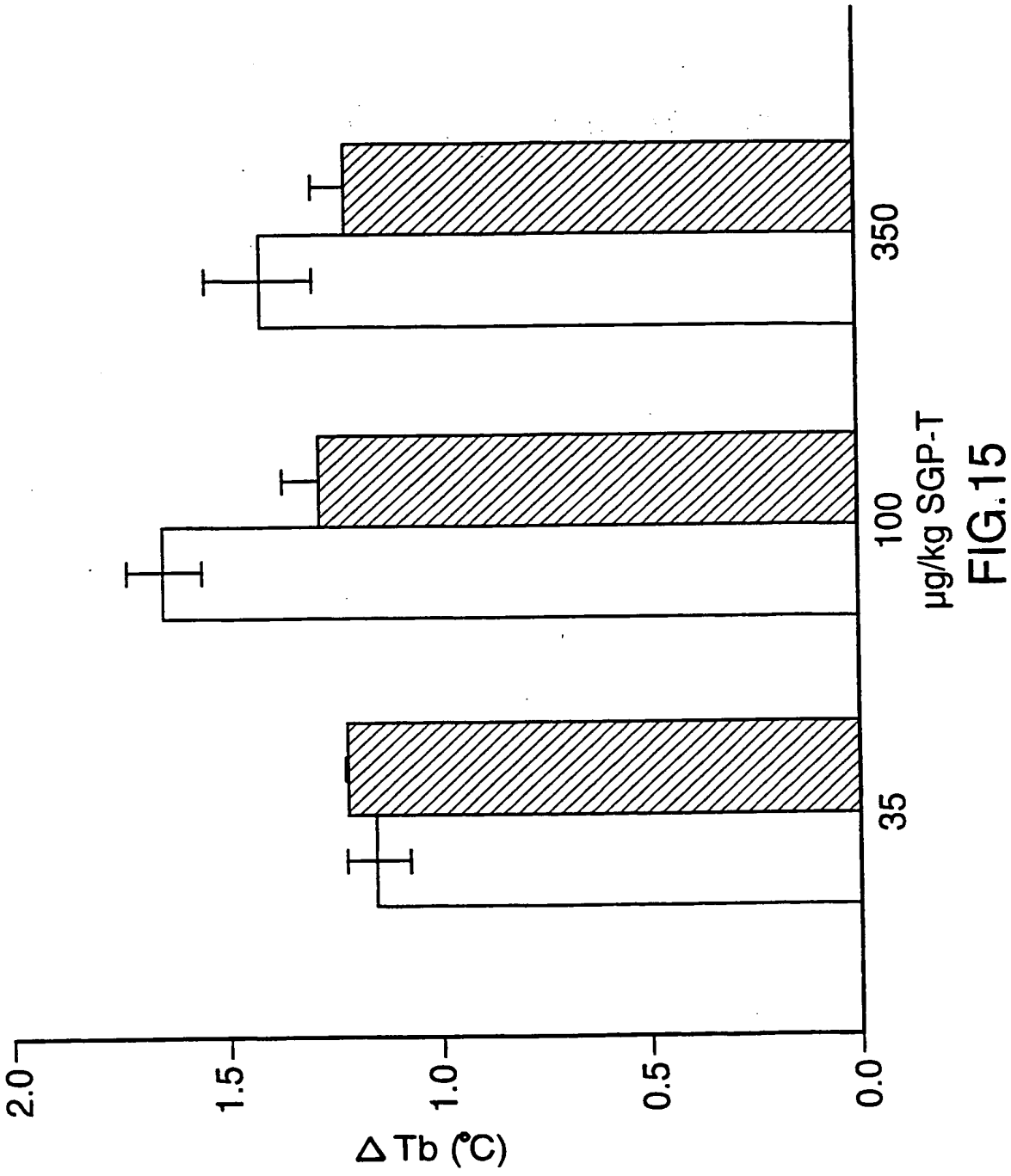

FIG.15

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